

**EXHIBIT 4**

**Applicants: Eran Blaugrund et al.**

**Serial No.: 10/712,958**

**Filed: November 13, 2003**



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT) -

(51) International Patent Classification <sup>6</sup> : <b>A61K 31/135</b>	<b>A1</b>	(11) International Publication Number: <b>WO 96/37199</b> (43) International Publication Date: 28 November 1996 (28.11.96)
<p>(21) International Application Number: PCT/US96/07465</p> <p>(22) International Filing Date: 22 May 1996 (22.05.96)</p> <p>(30) Priority Data: 08/446,439 22 May 1995 (22.05.95) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 08/446,439 (CON) Filed on 22 May 1995 (22.05.95)</p> <p>(71) Applicants (for all designated States except US): TEVA PHARMACEUTICAL INDUSTRIES, LTD. [IL/IL]; Science Based Industries Campus, Har Hotzvim, P.O. Box 1142, 91010 Jerusalem (IL). TECHNION RESEARCH AND DEVELOPMENT FOUNDATION LTD. [IL/IL]; Senate House, Technion City, 32000 Haifa (IL).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): YODIM, Moussa, B., H. [IL/IL]; 23A Remez, 36000 Tivon (IL). FINBERG, John, P., M. [IL/IL]; 15 Ben Zvi Street, 36000 Tivon (IL). LEVY, Ruth [IL/IL]; 7 Alterman Street, 69415 Tel Aviv (IL). STERLING, Jeffrey [US/IL]; Ramot 156/1, 97275</p>	<p>Jerusalem (IL). LERNER, David [US/IL]; 27/4 Yigal Street, 97241 Jerusalem (IL). YELLIN, Haim [IL/IL]; 45 Hayarden Street, 52333 Ramat-Gan (IL). VEINBERG, Alex [IL/IL]; 5 Aharoni Street, 76281 Rehovot (IL).</p> <p>(74) Agent: WHITE, John, P.; Cooper &amp; Dunham L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> With international search report.</p>	
(54) Title: USE OF R-ENANTIOMER OF N-PROPARGYL-1-AMINOINDAN, SALTS, AND COMPOSITIONS THEREOF		
(57) Abstract		
<p>The subject invention provides R(+)-N-propargyl-1-aminoindan and pharmaceutically acceptable salts thereof, as well as pharmaceutical compositions containing same. The subject invention also provides methods of treating a subject afflicted with Parkinson's disease, a memory disorder, dementia, depression, hyperactive syndrome, an effective illness, a neurodegenerative disease, a neurotoxic injury, stroke, brain ischemia, a head trauma injury, a spinal trauma injury, neurotrauma, schizophrenia, an attention deficit disorder, multiple sclerosis, or withdrawal symptoms, using R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt of the subject invention. The subject invention further provides a method of preventing nerve damage in a subject. Finally, the subject invention provides methods of preparing R(+)-N-propargyl-1-aminoindan, a salt thereof, and racemic N-propargyl-1-aminoindan.</p> <p>Applicants: Eran Blaugrund et al. Serial No.: 10/712,958 Filed: November 13, 2003 Exhibit 4</p>		

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USE OF R-ENANTIOMER OF N-PROPARGYL-1-AMINOINDAN,  
SALTS, AND COMPOSITIONS THEREOF

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10 Throughout this application, various references are referred to. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

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Background of the Invention

20 I.

The subject invention is in the field of selective irreversible inhibitors of the enzyme monoamine oxidase (hereinafter MAO) and provides the R(+) enantiomer of N-propargyl-1-aminoindan (also referred to herein as PAI)  
25 which is a selective irreversible inhibitor of the B-form of monoamine oxidase enzyme (hereinafter MAO-B). The subject invention also provides pharmaceutical compositions containing R(+)PAI which are particularly useful for the treatment of Parkinson's disease, a memory  
30 disorder, dementia, depression, hyperactive syndrome, an affective illness, a neurodegenerative disease, a neurotoxic injury, stroke, brain ischemia, a head trauma injury, a spinal trauma injury, neurotrauma, schizophrenia, an attention

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deficit disorder, multiple sclerosis, and withdrawal symptoms.

## II.

5 Parkinson's disease is widely considered to be the result of  
degradation of the pre-synaptic dopaminergic neurons in the  
brain, with a subsequent decrease in the amount of the  
neurotransmitter dopamine being released. Inadequate  
dopamine release, therefore, leads to the onset of  
10 disturbances of voluntary muscle control, which disturbances  
are symptomatic of Parkinson's disease.

Various methods of treating Parkinson's disease have been  
established and are currently in widespread use, including,  
15 for example, the administration of L-DOPA together with a  
decarboxylase inhibitor such as L-carbidopa or benserazide.  
The decarboxylase inhibitor protects the L-DOPA molecule  
from peripheral decarboxylation and thus ensures L-DOPA  
uptake by the remaining dopaminergic neurons in the striatum  
20 of the brain. Here, the L-DOPA is converted into dopamine  
resulting in increased levels of dopamine in these neurons.  
In response to physiological impulses, these neurons are  
therefore capable of releasing larger amounts of dopamine at  
levels which approximate the normal required levels. L-DOPA  
25 treatment thus alleviates the symptoms of the disease and  
contributes to the well-being of the patient.

However, L-DOPA treatment has its drawbacks, the main one  
being that its effectiveness is optimal only during the  
30 first few years of treatment. After this period, the  
clinical response diminishes and is accompanied by adverse  
side effects which include dyskinesia, fluctuation in  
efficacy throughout the day ("on-off effect") and  
psychiatric symptoms such as confusional states, paranoia,  
35 and hallucinations. This decrease in the effect of L-DOPA

treatment is attributed to a number of factors, including the natural progression of the disease, alteration in dopamine receptors as a consequence of increased dopamine production or increased levels of dopamine metabolites, and pharmacokinetic problems of L-DOPA absorption (reviewed by Youdim, et al., Progress in Medicinal Chemistry, 21, 138-167 (1984)).

In order to overcome the drawbacks of L-DOPA treatment, various treatments have been devised in which L-DOPA is combined with MAO inhibitors with the aim of reducing the metabolic breakdown of newly formed dopamine (see for example, Chiese, P., U.S. Patent No. 4,826,875, issued May 2, 1989).

MAO exists in two forms known as MAO-A and MAO-B which are selective for different substrates and inhibitors. For example, MAO-B more efficiently metabolizes substrates such as 2-phenylethylamine, and is selectively and irreversibly inhibited by (-)-deprenyl as described below.

It should be noted, however, that treatments combining L-DOPA with an inhibitor of both MAO-A and MAO-B are undesirable, as they lead to adverse side effects related to an increased level of catecholamines throughout the neuraxis. Furthermore, complete inhibition of MAO is also undesirable as it potentiates the action of sympathomimetic amines such as tyramine, leading to the so-called "cheese effect" (reviewed by Youdim et al., Handbook of Experimental Pharmacology, ed. by Trendelenburg and Weiner, Springer-Verlag, 90, ch. 3 (1988)). As MAO-B was shown to be the predominant form of MAO in the brain, selective inhibitors for this form are thus considered to be a possible tool for achieving a decrease in dopamine breakdown on the one hand, together with a minimization of the systemic effects of

total MAO inhibition on the other.

Many inhibitors of MAO are chiral molecules. Although one enantiomer often shows some stereoselectivity in relative potency towards MAO-A and -B, a given enantiomeric configuration is not always more selective than its mirror image isomer in discriminating between MAO-A and MAO-B.

Table I lists the  $IC_{50}$  (mmol/L) of enantiomeric pairs of propargyl amines in a rat brain preparation of MAO. These results show small differences in potency in MAO-B inhibition between the R and S enantiomers. (B. Hazelhoff, et al., Naunyn-Schmeideberg's Arch. Pharmacol., 330, 50 (1985)). Both enantiomers are selective for MAO-B. In 1967, Magyar, et al. reported that R-(-)-deprenyl is 500 times more potent than the S-(+) enantiomer in inhibiting the oxidative deamination of tyramine by rat brain homogenate. (K. Magyar, et al., Act. Physiol. Acad. Sci., Hung., 32, 377 (1967)).

In rat liver homogenate, R-deprenyl is only 15 times as potent as the S enantiomer. In other pharmacological activity assays, such as for the inhibition of tyramine uptake, deprenyl shows different stereoselectivities. The S form is in certain cases the more potent epimer. (J. Knoll and K. Magyar, Advances in Biochemical Psychopharmacology, 5, 393 (1972)).

N-Methyl-N-propargyl-1-aminotetralin (2-MPAT) is a close structural analogue of deprenyl. The absolute stereochemistry of 2-MPAT has not been assigned. However, the (+) isomer is selective for MAO-B and the (-) isomer is selective for MAO-A. The difference in potency between the 2-MPAT enantiomers is less than 5-fold. (B. Hazelhoff, et al., id.). The enantiomers of N-propargyl-1-aminotetralin

(1-PAT) are also similar in activity. The lack of data in Table I showing clear structure-activity relationships between isolated (+) or (-)-2-MPAT makes it impossible to predict the absolute stereochemistry thereof.

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After extensive computer modeling, Polymeropoulos recently predicted that (R)-N-methyl-N-propargyl-1-aminoindan (R-1-MPAI) would be more potent than (S) as a MAO-B inhibitor. (E. Polymeropoulos, Inhibitors of Monoamine Oxidase B, I. Szelenyi, ed., Birkhauser Verlag, p. 110 (1993)). However, experiments described show that R-1-MPAI is a slightly more potent inhibitor of MAO-B than S-1-MPAI, but is an even more potent inhibitor of MAO-A. Both the selectivity between MAO-A and -B and the relative potency of the R and S epimers are low. Thus, contrary to expectations in the art, 1-MPAI is useless as a pharmaceutical agent.

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The data presented below demonstrate that high selectivity for MAO of one enantiomer versus the other cannot be predicted. The structure of the MAO active site is not well enough understood to permit the prediction of the relative potency or selectivity of any given compound or pair of enantiomers thereof.

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### III.

Brain stroke is the third leading cause of death in the developed countries. Survivors often suffer from neurological and motor disabilities. The majority of CNS strokes are regarded as localized tissue anemia following obstruction of arterial blood flow which causes oxygen and glucose deprivation. Occlusion of the middle cerebral artery in the rat (MCAO) is a common experimental procedure that is assumed to represent stroke in humans. It has been proposed that the neurological lesion caused by proximal occlusion of this artery in the rat corresponds to a large

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focal cerebral infarct in humans (Yamori et al., 1976). This correspondence has been based on similarities between cranial circulation in the two species. Other animal models of stroke have been described by Stefanovich (1983).

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The histological changes described by Tamura et al. (1981) who were the first to introduce the MCAO procedure, were commonly seen in the cortex of the frontal (100%), sensimotor (75%) and auditory (75%) areas and to a lesser extent in the occipital lobe cortex (25%). In addition, damage was observed in the lateral segment of the caudate nucleus (100%), and only to a variable extent in its medial portion (38%). Concomitantly, the following disorders were reported in MCAO animals: neurological deficits (Menziés et al., 1992), cognitive disturbances (Yamamoto et al., 1988), brain edema (Young et al., 1993; Matsui et al., 1993; Saur et al., 1993), decreased cerebral blood flow (Teasdale et al., 1983), catecholamine fluctuations. (Cechetto et al., 1989). Any of these disorders might be indicative of the severity and extent of brain damage that follow MCAO in the rat. Conversely, a drug with a potential to limit or abort a given disorder may be considered as a candidate for the treatment of stroke in humans.

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Table IA IC<sub>50</sub> (mmol/L) Data for Rat Brain MAO Inhibition by Propargylamines

COMPOUND	REF	EPIMER	INHIBITION		RELATIVE POTENCY A/B	POTENCY +/-	
			A	B		A	B
2-MPAI	a	+	140	16	8.8	3	0.2
		-	46	88	0.5		
DEPRENYL	a	S	3600	16	120	R/S	
		R	450	6	75	80	2.6
1-MPAI	b	S	70	50	1.4	23	5
		R	3	10	0.3		
1-PAT	c	S	3800	50	76	4	0.5
		R	900	90	10		

- a. B. Hazelhoff, et al., Naunyn-Schmeideberg's Arch. Pharmacol., 330, 50 (1985).
- b. European Patent Application 436,492 A2, published July 10, 1991.
- c. Present inventors.

One selective MAO-B inhibitor, (-)-deprenyl, has been extensively studied and used as a MAO-B inhibitor to augment L-DOPA treatment. This treatment with (-)-deprenyl is generally favorable and does not cause the "cheese effect" at doses causing nearly complete inhibition of MAO-B

(Elsworth, et al., Psychopharmacology, 57, 33 (1978)). Furthermore, the addition of (-)-deprenyl to a combination of L-DOPA and a decarboxylase inhibitor administered to Parkinsons's patients leads to improvements in akinesia and overall functional capacity, as well as the elimination of "on-off" type fluctuations (reviewed by Birkmayer & Riederer in "Parkinson's Disease," Springer-Verlag, pp. 138-149 (1983)). Thus, (-)-deprenyl (a) enhances and prolongs the effect of L-DOPA, and (b) does not increase the adverse effects of L-DOPA treatment.

However, (-)-deprenyl is not without its own adverse sides effects, which include activation of pre-existing gastric ulcers and occasional hypertensive episodes. Furthermore, (-)-deprenyl is an amphetamine derivative and is metabolized to amphetamine and methamphetamines, which substances may lead to undesirable side effects such as increased heart rate (Simpson, Biochemical Pharmacology, 27, 1951 (1978); Finberg, et al., in "Monoamine Oxidase Inhibitors - The State of the Art," Youdim and Paykel, eds., Wiley, pp. 31-43 (1981)).

Other compounds have been described that are selective irreversible inhibitors of MAO-B but which are free of the undesirable effects associated with (-)-deprenyl. One such compound, namely N-propargyl-1-aminoindan·HCl (racemic PAI·HCl), was described in GB 1,003,686 and GB 1,037,014 and U.S. Patent No. 3,513,244, issued May 19, 1970. Racemic PAI·HCl is a potent, selective, irreversible inhibitor of MAO-B, is not metabolized to amphetamines, and does not give rise to unwanted sympathomimetic effects.

In comparative animal tests, racemic PAI was shown to have considerable advantages over (-)-deprenyl. For example, racemic PAI produces no significant tachycardia, does not

increase blood pressure (effects produced by doses of 5mg/kg of (-)-deprenyl), and does not lead to contraction of nictitating membrane or to an increase in heart rate at doses of up to 5mg/kg (effects caused by (-)-deprenyl at  
5 doses over 0.5 mg/kg). Furthermore, racemic PAI·HCl does not potentiate the cardiovascular effects of tyramine (Finberg, et al., in "Enzymes and Neurotransmitters in Mental Disease," pp. 205-219 (1980), Usdin, et al., Eds., Wiley, New York; Finberg, et al. (1981), in "Monoamine  
10 Oxidase Inhibitors - The State of the Art," ibid.; Finberg and Youdim, British Journal Pharmacol., 85, 451 (1985)).

One underlying object of this invention was to separate the racemic PAI compounds and to obtain an enantiomer with MAO-B  
15 inhibition activity which would be free of any undesirable side effects associated with the other enantiomer.

Since deprenyl has a similar structure to PAI and it is known that the (-)-enantiomer of deprenyl, i.e. (-)-  
20 deprenyl, is considerably more pharmaceutically active than the (+)-enantiomer, the (-) enantiomer of PAI would be expected to be the more active MAO-B inhibitor.

However, contrary to such expectations, upon resolution of the enantiomers, it was found that the (+)-PAI enantiomer is  
25 in fact the active MAO-B inhibitor while the (-)-enantiomer shows extremely low MAO-B inhibitory activity. Furthermore, the (+)-PAI enantiomer also has a degree of selectivity for MAO-B inhibition surprisingly higher than that of the  
30 corresponding racemic form, and should thus have fewer undesirable side effects in the treatment of the indicated diseases than would the racemic mixture. These findings are based on both in vitro and in vivo experiments as discussed in greater detail infra.

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It was subsequently shown that (+)-PAI has the R absolute configuration. This finding was also surprising based on the expected structural similarity of (+)-PAI analogy with deprenyl and the amphetamines.

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The high degree of stereoselectivity of pharmacological activity between R(+)-PAI and the S(-) enantiomer as discussed hereinbelow is also remarkable. The compound R(+)-PAI is nearly four orders of magnitude more active than the S(-) enantiomer in MAO-B inhibition. This ratio is significantly higher than that observed between the two deprenyl enantiomers (Knoll and Magyar, Adv. Biochem. Psychopharmacol., 5, 393 (1972); Magyar, et al., Acta Physiol. Acad. Sci. Hung., 32, 377 (1967)). Furthermore, in some physiological tests, (+)-deprenyl was reported to have activity equal to or even higher than that of the (-) enantiomer (Tekes, et al., Pol. J. Pharmacol. Pharm., 40, 653 (1988)).

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MPAI is a more potent inhibitor of MAO activity, but with lower selectivity for MAO-B over A (Tipton, et al., Biochem. Pharmacol., 31, 1250 (1982)). As only a small degree of difference in the relative activities of the two resolved enantiomers was surprisingly observed with MPAI, the remarkable behavior of R(+)-PAI is further emphasized (See Table 1B).

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The subject invention also provides methods of using the pharmaceutically active PAI-enantiomer alone (without L-DOPA) for treatment of Parkinson's disease, a memory disorder, dementia, depression, hyperactive syndrome, an affective illness, a neurodegenerative disease, a neurotoxic injury, brain ischemia, a head trauma injury, a spinal trauma injury, schizophrenia, an attention deficit disorder, multiple sclerosis, or withdrawal symptoms (see review by

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Youdim, et al., in Handbook of Experimental Pharmacology, Trendelenberg and Wiener, eds., 90/I, ch. 3 (1988)).

5 The subject invention further provides a method of using the  
pharmaceutically active PAI-enantiomer alone for pre-  
treatment of Parkinson's disease. The subject invention  
also provides pharmaceutical compositions comprising R(+)PAI  
and synergistic agents such as levodopa. The use of such  
10 agents has been studied with respect to (-)-deprenyl which  
was shown to be effective when administered alone to early  
Parkinson's patients, and may also have a synergistic effect  
in these patients when administered together with  $\alpha$ -  
tocopherol, a vitamin E derivative (The Parkinson's Study  
Group, New England J. Med., 321(20), 1364-1371 (1989)).

15 In addition to its usefulness in treating Parkinson's  
disease, (-)-deprenyl has also been shown to be useful in  
the treatment of patients with dementia of the Alzheimer  
type (DAT) (Tariot, et al., Psychopharmacology, 91, 489-495  
20 (1987)), and in the treatment of depression (Mendelewicz and  
Youdim, Brit. J. Psychiat. 142, 508-511 (1983)). The  
R(+)PAI compound of this invention, and particularly the  
mesylate salt thereof, has been shown to restore memory.  
R(+)PAI thus has potential for the treatment of memory  
25 disorders, dementia, especially of the Alzheimer's type, and  
hyperactive syndrome in children.

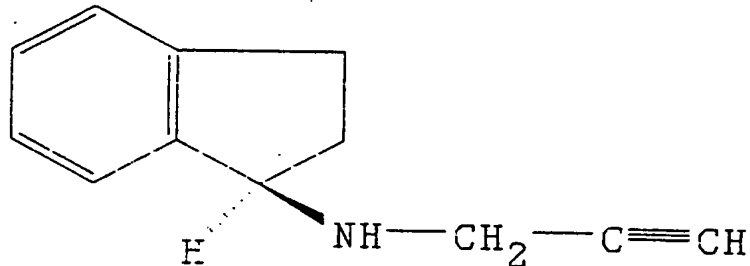
Finally, the subject invention provides highly stable salts  
of R(+)PAI with superior pharmaceutical properties. The  
30 mesylate salt is especially stable, shows unexpectedly  
greater selectivity, and shows significantly fewer side  
effects than do the corresponding racemic salts.

Summary of the Invention

The subject invention provides R(+)-N-propargyl-1-aminoindan having the structure:

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The subject invention further provides a pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan.

The subject invention further provides a pharmaceutical composition which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

The subject invention further provides a method of treating a subject afflicted with Parkinson's disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat Parkinson's disease in the subject.

The subject invention further provides a method of treating a subject afflicted with a memory disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the memory disorder in the subject.

5 The subject invention further provides a method of treating a subject afflicted with dementia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat dementia in the subject. In one embodiment, the dementia is of the Alzheimer type (DAT).

10 The subject invention further provides a method of treating a subject afflicted with depression which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat depression in the subject.

15 The subject invention further provides a method of treating a subject afflicted with hyperactive syndrome which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat hyperactive syndrome in the subject.

20 The subject invention further provides a method of treating a subject afflicted with an affective illness which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the affective illness in the subject.

25 The subject invention further provides a method of treating a subject afflicted with a neurodegenerative disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the neurodegenerative disease in the subject.



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The subject invention further provides a method of treating a subject afflicted with a neurotoxic injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the neurotoxic injury in the subject.

The subject invention further provides a method of treating a subject afflicted with brain ischemia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat brain ischemia in the subject.

The subject invention further provides a method of treating a subject afflicted with a head trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the head trauma injury in the subject.

The subject invention further provides a method of treating a subject afflicted with a spinal trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the spinal trauma injury in the subject.

The subject invention further provides a method of treating a subject afflicted with schizophrenia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat schizophrenia in the subject.

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5 The subject invention further provides a method of treating a subject afflicted with an attention deficit disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the attention deficit disorder in the subject.

10 The subject invention further provides a method of treating a subject afflicted with multiple sclerosis which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat multiple sclerosis in the subject.

15 The subject invention further provides a method of preventing nerve damage in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to prevent nerve damage in the subject.

25 The subject invention further provides a method of treating a subject suffering from symptoms of withdrawal from an addictive substance which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the symptoms of withdrawal in the subject.

30 The subject invention further provides a method for preparing R(+)-N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, R(-)-aminoindan with either propargyl bromide or propargyl chloride so as to form R(+)-N-propargyl-1-aminoindan, and  
35 isolating the R(+)-N-propargyl-1-aminoindan formed thereby.

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5 The subject invention further provides a method for preparing racemic N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, racemic 1-aminoindan with propargyl bromide or propargyl chloride so as to form racemic N-propargyl-1-aminoindan, and isolating the racemic N-propargyl-1-aminoindan formed thereby.

10 Finally, the subject invention provides a method of preparing an R(+)-N-propargyl-1-aminoindan salt which comprises contacting racemic N-propargyl-1-aminoindan with an optically active acid so as to form two diastereomeric N-propargyl-1-aminoindan salts, and isolating R(+)-N-propargyl-1-aminoindan salt from the diastereomeric N-  
15 propargyl-1-aminoindan salts so formed.

Brief Description of the Figures

5     Figure 1 is a graphic representation of the results according to Example 22 showing in vitro MAO-A inhibitory activity.

10    Figure 2 is a graphic representation of the results according to Example 22 showing in vitro MAO-B inhibitory activity.

15    Figure 3 is a graphic representation of the results according to Example 22 showing MAO activity in human cortical tissue.

20    Figure 4 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-A in brain.

25    Figure 5 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-B in brain.

30    Figure 6 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-A in liver.

35    Figure 7 is a graphic representation of the results according to Example 23 showing acute inhibition (i.p.) of MAO-B in liver.

40    Figure 8 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-A in brain.

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Figure 9 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-B in brain.

5     Figure 10 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-A in liver.

10    Figure 11 is a graphic representation of the results according to Example 23 showing acute inhibition (per os) of MAO-B in liver.

15    Figure 12 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-A in brain.

20    Figure 13 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-B in brain.

25    Figure 14 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-A in liver.

30    Figure 15 is a graphic representation of the results according to Example 24 showing chronic inhibition of MAO-B in liver.

35    Figure 16 is a graphic representation of the results according to Example 25 showing MAO-B activity in rat brain as a function of time following i.p. administration of R(+)PAI.

Figure 17 is a graphic representation of the results according to Example 32 showing restoration of normokinesia in mice that had received haloperidol 6 mg/kg s.c. Mice

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received each of the test drugs i.p. at the indicated dose. 2 hours later they received haloperidol. Kinetic scores were taken 3 hours after haloperidol. These scores consisted of the ability to move horizontally along a rod, the ability to descend a vertical rod, and the shortening of catalepsia. In the absence of haloperidol, the maximum score is 12; with haloperidol alone,  $6.6 \pm 0.03$ . Statistical significance was calculated by the Student's "t" test: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$  with respect to haloperidol alone. The scores of (R)-PAI are significantly different from those of racemic-PAI at 5 mg/kg ( $p \leq 0.05$ ), at 10 mg/kg ( $p \leq 0.01$ ), and at 15 mg/kg ( $p \leq 0.05$ ), ( $n=5.6$ ). The dosage shown is for the free base of PAI (and not the mesylate salt).

Figure 18 is a graphic representation of the results according to Example 32 showing restoration of motor activity in rats treated with  $\alpha$ -methyl-p-tyrosine at 100 mg/kg i.p. Rats received the test drug i.p. at the indicated doses. After two hours they received  $\alpha$ -Mpt and were immediately placed in activity cages. Total motor activity was recorded for the duration of 10 hours. Control rats, treated with saline, only scored  $15,862 \pm 1424$ . With  $\alpha$ -Mpt alone, they scored  $8,108 \pm 810$ . Statistical significance by the Student's "t" test: \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$  with respect to  $\alpha$ -Mpt alone. The scores of (R)-PAI are significantly different from racemic-PAI at 2 mg/kg ( $p \leq 0.01$ ), ( $n=6$ ). Dosage shown is for the free base of PAI and not the mesylate salt.

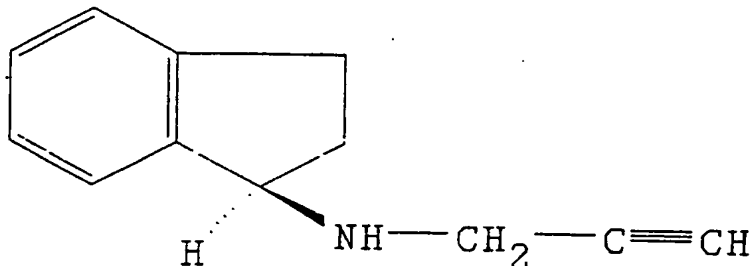
Figure 19 is a graph showing the NADH response to 2 minutes of anoxia measured 30 minutes after injury and at half-hour intervals thereafter.

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- Figure 20: Ischemic brain lesion evaluation with MRI T2-scan 48 hours after MCA-O and [R] (+)PAI Mesylate Treatment in rats: The middle cerebral artery was surgically occluded as described in Example 38. [R] (+)PAI Mesylate was administered as follows: 1.0 mg/kg ip immediately after surgery; 0.5 mg/kg ip, 2 hrs after surgery; 1.0 mg/kg ip, 24 hrs after surgery. Infarct volume (mm<sup>3</sup>) was determined by MRI 48 hours following surgery.
- Figure 21: Neurological evaluation of Wistar rats subjected to MCA-O and [R] (+)PAI Mesylate Treatment: The middle cerebral artery was surgically occluded and [R] (+)PAI Mesylate administered as in Figure 20. At 24 hours post surgery a neurological score was taken as described in Example 38.

Detailed Description of the Invention

The subject invention provides R(+)-N-propargyl-1-aminoindan having the structure:



As demonstrated in the Experimental Examples hereinbelow, R(+)-PAI is nearly 7,000 times more active as an inhibitor of MAO-B than is S(-)-PAI. In view of known MAO-B inhibitors in the art which possess low selectivity between MAO-A and MAO-B, and which do not show predictable trends in potency as a function of R or S configuration, the selectivity of R(+)-PAI is unexpected.

R(+)-PAI may be obtained by optical resolution of racemic mixtures of R- and S-enantiomers of PAI. Such a resolution can be accomplished by any conventional resolution method well known to a person skilled in the art, such as those described in J. Jacques, A. Collet and S. Wilen, "Enantiomers, Racemates and Resolutions," Wiley, New York (1981). For example, the resolution may be carried out by preparative chromatography on a chiral column. Another example of a suitable resolution method is the formation of diastereomeric salts with a chiral acid such as tartaric, malic, mandelic acid or N-acetyl derivatives of amino acids, such as N-acetyl leucine, followed by recrystallisation to isolate the diastereomeric salt of the desired R enantiomer.



The racemic mixture of R and S enantiomers of PAI may be prepared, for example, as described in GB 1,003,676 and GB 1,037,014. The racemic mixture of PAI can also be prepared by reacting 1-chloroindan with propargylamine. Alternatively, this racemate may be prepared by reacting propargylamine with 1-indanone to form the corresponding imine, followed by reduction of the carbon-nitrogen double bond of the imine with a suitable agent, such as sodium borohydride.

In accordance with this invention, the R enantiomer of PAI can also be prepared directly from the optically active R-enantiomer of 1-aminoindan by reaction with propargyl bromide or propargyl chloride in the presence of an organic or inorganic base, and optionally in the presence of a suitable solvent.

Suitable organic or inorganic bases for use in the above reaction include, by way of example, triethylamine, pyridine, alkali metal carbonates, and bicarbonates. If the reaction is conducted in the presence of a solvent, the solvent may be chosen from, e.g., toluene, methylene chloride, and acetonitrile. One method of preparing R(+)PAI is to react R-1-aminoindan with propargyl chloride using potassium bicarbonate as a base and acetonitrile as solvent.

The above-described reaction of 1-aminoindan generally results in a mixture of unreacted primary amine, the desired secondary amine and the tertiary amine N,N-bispropargylamino product. The desired secondary amine, i.e., N-propargyl-1-aminoindan, can be separated from this mixture by a conventional separation method including, by way of example, chromatography, distillation and selective extraction.

The R-1-aminoindan starting material can be prepared by methods known in the art which include, by way of example, the method of Lawson and Rao, Biochemistry, 19, 2133 (1980), methods in references cited therein, and the method of  
5 European Patent No. 235,590.

R-1-aminoindan can also be prepared by resolution of a racemic mixture of the R and S enantiomers, which involves, for example, the formation of diastereomeric salts with  
10 chiral acids, or any other known method such as those reported in J. Jacques, et al., ibid. Alternatively, R-1-aminoindan may be prepared by reacting 1-indanone with an optically active amine, followed by reduction of the carbon nitrogen double bond of the resulting imine by hydrogenation  
15 over a suitable catalyst, such as palladium on carbon, platinum oxide or Raney nickel. Suitable optically active amines include, for example, one of the antipodes of phenethylamine or an ester of an amino acid, such as valine or phenylalanine. The benzylic N-C bond may be cleaved  
20 subsequently by hydrogenation under non-vigorous conditions.

An additional method for preparing R-1-aminoindan is the hydrogenation of indan-1-one oxime ethers as described above, wherein the alkyl portion of the ether contains an  
25 optically pure chiral center. Alternatively, a non-chiral derivative of indan-1-one containing a carbon-nitrogen double bond, such as an imine or oxime, can be reduced with a chiral reducing agent, e.g., a complex of lithium aluminum-hydride and ephedrine.

30 The subject invention further provides a pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan.

In the practice of this invention, pharmaceutically  
35 acceptable salts include, but are not limited to, the

mesylate, maleate, fumarate, tartrate, hydrochloride, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate and sulfate salts.

5 In one embodiment, the salt is selected from the group consisting of the mesylate salt of R(+)-N-propargyl-1-aminoindan, the esylate salt of R(+)-N-propargyl-1-aminoindan, and the sulfate salt of R(+)-N-propargyl-1-aminoindan.

10

As demonstrated in the Experimental Examples hereinbelow, the mesylate salt is highly stable to thermal degradation, and shows unexpectedly superior selectivity for MAO-B over the racemic salt.

15

For the preparation of pharmaceutically acceptable acid addition salts of the compound of R(+)PAI, the free base can be reacted with the desired acids in the presence of a suitable solvent by conventional methods. Similarly, an acid addition salt may be converted to the free base form in a known manner.

20 A preferred mode of preparing the mesylate salt of (R)-PAI comprises (a) adding an aqueous solution of 15% sodium hydroxide to a solution of propargyl benzenesulfonate (or tosylate or mesylate) in toluene; (b) stirring for 5 hours; (c) adding additional toluene and water; (d) separating and washing the organic phase with 10% sodium hydroxide, and then diluting with water; (e) adjusting the pH of the mixture to 3.2 by adding 10% aqueous sulfuric acid; (f) separating the aqueous phase and adjusting the pH to 7.3 with 10% sodium hydroxide; (g) extracting three times with toluene while maintaining constant pH; (h) concentrating combined organic layers in vacuo to give a yellow oil; (i) dissolving the oil and L-tartaric acid in isopropanol; (j)

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heating to reflux for 1 hour; (k) cooling to room temperature and collecting the precipitate by filtration; (l) recrystallizing the crude di-propargylaminoindan tartrate from methanol/isopropanol (1:1) to give di(R(+)-N-propargyl-1-aminoindan) tartrate; (m) dissolving the tartrate salt and methanesulfonic acid in isopropanol, and heating to reflux for 30 minutes; and (n) cooling to room temperature, and collecting the precipitated R(+)-N-propargyl-1-aminoindan.

The subject invention further provides a pharmaceutical composition which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. The "therapeutically effective amount" of the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof may be determined according to methods well known to those skilled in the art.

Possible salts useful for such compositions include hydrochloride, phosphate, maleate, fumarate, tartrate, mesylate, esylate, and sulfate salts.

These compositions may be prepared as medicaments to be administered orally, parenterally, rectally, or transdermally.

In one embodiment, the pharmaceutically acceptable carrier is a solid and the pharmaceutical composition is a tablet. The therapeutically effective amount may be an amount from about 0.1 mg to about 100 mg. The therapeutically effective amount may also be an amount from about 1 mg to about 10 mg.

Suitable forms for oral administration include tablets, compressed or coated pills, dragees, sachets, hard or soft

gelatin capsules, sublingual tablets, syrups and suspensions.

5 In an alternative embodiment, the pharmaceutically acceptable carrier is a liquid and the pharmaceutical composition is an injectable solution. The therapeutically effective amount may be an amount from about 0.1 mg/ml to about 100 mg/ml. The therapeutically effective amount may also be an amount from about 1 mg/ml to about 10 mg/ml. In 10 one embodiment, the dose administered is an amount between 0.5ml and 1.0ml.

15 In a further alternative embodiment, the carrier is a gel and the pharmaceutical composition is a suppository.

For parenteral administration the invention provides ampoules or vials that include an aqueous or non-aqueous solution or emulsion. For rectal administration there are provided suppositories with hydrophilic or hydrophobic 20 vehicles. For topical application as ointments and transdermal delivery there are provided suitable delivery systems as known in the art.

25 In the preferred embodiment, the pharmaceutically acceptable salt is a mesylate salt.

30 These compositions may be used alone to treat the above-listed disorders, or alternatively, as in the case of Parkinson's disease, for example, they may be used as an adjunct to the conventional L-DOPA treatments.

The preferred dosages of the active ingredient, i.e., R-PAI, in the above compositions are within the following ranges. For oral or suppository formulations, 0.1-100 mg per dosage 35 unit may be taken daily, and preferably 1-10 mg per dosage

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unit is taken daily. For injectable formulations, 0.1-100 mg/ml per dosage unit may be taken daily, and preferably 1-10 mg/ml per dosage unit is taken daily.

5 In one embodiment, the pharmaceutical composition further comprises a therapeutically effective amount of Levodopa. In another embodiment, the pharmaceutical composition still further comprises an effective amount of a decarboxylase inhibitor.

10

The amount of decarboxylase inhibitor administered in combination with (R)-PAI or a pharmaceutically acceptable salt thereof is an amount effective to ensure L-DOPA uptake in the subject.

15

The decarboxylase inhibitor may be L-Carbidopa. In one embodiment, the therapeutically effective amount of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the therapeutically effective amount of Levodopa is about 50 mg  
20 to about 250 mg, and the effective amount of L-Carbidopa is about 10 mg to about 25 mg.

The decarboxylase inhibitor may also be benserazide. In one embodiment, the therapeutically effective amount of R(+)-N-propargyl-1-aminoindan is about 0.1 mg to about 100 mg, the  
25 therapeutically effective amount of Levodopa is about 50 mg to about 200 mg, and the effective amount of benserazide is about 12.5 mg to about 50 mg.

30 The subject invention further provides a method of treating a subject afflicted with Parkinson's disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat Parkinson's  
35 disease in the subject.

Methods of treatment of Parkinson's disease which combine the use of (R)-PAI with other drugs, such as dopamine agonists, bromocryptine, pergolide, lisuride, as well as catecholamine oxidase methyl transferase inhibitors are  
5 within the scope of the subject invention.

In the preferred embodiment, the pharmaceutically acceptable salt is a mesylate salt.

10 The administering may comprise orally administering, rectally administering, transdermally administering, or parenterally administering.

In one embodiment, the method of the subject invention  
15 further comprises administering to the subject a therapeutically effective amount of Levodopa. In another embodiment, the method of the subject invention still further comprises administering to the subject an effective amount of a decarboxylase inhibitor.

20 The decarboxylase inhibitor may be L-Carbidopa. Alternatively, the decarboxylase inhibitor may be benserazide.

25 The subject invention further provides a method of treating a subject afflicted with a memory disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof  
30 of the subject invention effective to treat the memory disorder in the subject.

The subject invention further provides a method of treating a subject afflicted with dementia which comprises  
35 administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof

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of the subject invention effective to treat dementia in the subject. In one embodiment, the dementia is of the Alzheimer type (DAT).

5 The subject invention further provides a method of treating a subject afflicted with depression which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat depression in  
10 the subject.

The subject invention further provides a method of treating a subject afflicted with hyperactive syndrome which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable  
15 salt thereof of the subject invention effective to treat hyperactive syndrome in the subject.

The administering may comprise orally administering, rectally administering, or parenterally administering.  
20

The subject invention further provides a method of treating a subject afflicted with an affective illness which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable  
25 salt thereof of the subject invention effective to treat the affective illness in the subject.

The subject invention further provides a method of treating a subject afflicted with a neurodegenerative disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable  
30 salt thereof of the subject invention effective to treat the neurodegenerative disease in the subject.

35



The subject invention further provides a method of treating a subject afflicted with a neurotoxic injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof  
5 of the subject invention effective to treat the neurotoxic injury in the subject.

The subject invention further provides a method of treating a subject afflicted with brain ischemia which comprises  
10 administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat brain ischemia in the subject.

15 This invention provides a method of treating brain ischemia or stroke in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat brain ischemia or stroke in the subject.

20 In an embodiment of the method for treatment of brain ischemia or stroke, the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of: the mesylate salt; the ethylsulfonate salt;  
25 the sulfate salt; and the hydrochloride salt. Preferably, the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.

30 The effective amount can be determined using techniques known to those of skill in the art, such as titration. In an embodiment of this invention, the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of the subject. The R(+)-N-propargyl-1-aminoindan or  
35 pharmaceutically acceptable salt thereof is administered

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using techniques known to those of skill in the art. For example, it may be administered intravenously, orally, rectally, transdermally, or parenterally.

5 The subject is preferably a mammal, such as a dog, cat, mouse, rat, rabbit, pig, horse, goat, sheep, cow, ape or monkey. In a particular embodiment the subject is human.

10 In an embodiment of this invention, the effective amount is from about 0.01 mg to 50.0 mg per day. In a more specific embodiment, the effective amount is from 0.1 to 10.0 mg per day.

15 In one embodiment of the above-described method, the area of the brain ischemia is reduced by about thirty-five percent.

20 The subject invention further provides a method of treating a subject afflicted with a head trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the head trauma injury in the subject.

25 The subject invention further provides a method of treating a subject afflicted with a spinal trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the spinal trauma injury in the subject.

30 This invention further provides a method of treating neurotrauma in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat  
35 neurotrauma in the subject.

5 In the treatment of head trauma injury, spinal trauma injury or neurotrauma, the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of: the mesylate salt; the ethylsulfonate salt; the sulfate salt; and the hydrochloride salt. Preferably, the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.

10 The effective amount can be determined using techniques known to those of skill in the art, such as titration. In an embodiment of this invention, the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of  
15 the subject. The R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered using techniques known to those of skill in the art. For example, it may be administered intravenously, orally, rectally, transdermally, or parenterally.

20 The subject is preferably a mammal, such as a dog, cat, mouse, rat, rabbit, pig, horse, goat, sheep, cow, ape or monkey. In a particular embodiment the subject is human.

25 In an embodiment of this invention, the effective amount is from about 0.01 mg to 50.0 mg per day. In a more specific embodiment, the effective amount is from 0.1 to 10.0 mg per day.

30

The subject invention further provides a method of treating  
35 a subject afflicted with schizophrenia which comprises administering to the subject an amount of R(+)-N-propargyl-

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1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat schizophrenia in the subject.

5 The subject invention further provides a method of treating a subject afflicted with an attention deficit disorder which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat the  
10 attention deficit disorder in the subject.

The subject invention further provides a method of treating a subject afflicted with multiple sclerosis which comprises administering to the subject an amount of R(+)-N-propargyl-  
15 1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to treat multiple sclerosis in the subject.

The subject invention further provides a method of  
20 preventing nerve damage in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject invention effective to prevent nerve damage in the subject.

25 In one embodiment, the nerve damage is structural nerve damage. In another embodiment, the structural nerve damage is optic nerve damage.

30 The subject invention further provides a method of treating a subject suffering from symptoms of withdrawal from an addictive substance which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or the pharmaceutically acceptable salt thereof of the subject  
35 invention effective to treat the symptoms of withdrawal in

the subject.

As used herein, the term "symptoms of withdrawal" refers to physical and/or psychological symptoms, including drug  
5 craving, depression, irritability, anergia, amotivation, appetite change, nausea, shaking and sleep irregularity.

As used herein, the term "addictive substance" includes, by way of example, (a) addictive opiates such as opium, heroin  
10 and morphine, (b) psychostimulants such as cocaine, amphetamines and methamphetamines, (c) alcohol, (d) nicotine, (e) barbiturates and (f) narcotics such as fentanyl, codeine, diphenoxylate and thebaine.

15 In one embodiment, the addictive substance is cocaine. In another embodiment, the addictive substance is alcohol.

The subject invention further provides a method for preparing R(+)-N-propargyl-1-aminoindan which comprises  
20 contacting, in the presence of an organic or inorganic base, R(-)-aminoindan with either propargyl bromide or propargyl chloride so as to form R(+)-N-propargyl-1-aminoindan, and isolating the R(+)-N-propargyl-1-aminoindan formed thereby.

25 The subject invention further provides a method for preparing racemic N-propargyl-1-aminoindan which comprises contacting, in the presence of an organic or inorganic base, racemic 1-aminoindan with propargyl bromide or propargyl  
30 chloride so as to form racemic N-propargyl-1-aminoindan, and isolating the racemic N-propargyl-1-aminoindan formed thereby.

Finally, the subject invention provides a method of preparing an R(+)-N-propargyl-1-aminoindan salt which  
35 comprises contacting racemic N-propargyl-1-aminoindan with

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an optically active acid so as to form two diastereomeric N-propargyl-1-aminoindan salts, and isolating R(+)-N-propargyl-1-aminoindan salt from the diastereomeric N-propargyl-1-aminoindan salts so formed.

5

In one embodiment, the isolating comprises isolating by fractional crystallization.

10

The following Experimental Details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims which follow thereafter.

Experimental DetailsEXAMPLE 15     Racemic N-propargyl-1-aminoindan hydrochloride

10.0g of racemic 1-aminoindan and 10.4g of potassium carbonate were added to 75 ml of acetonitrile. The resulting suspension was heated to 60°C and 4.5g of propargyl chloride was added dropwise.

The mixture was stirred at 60°C for 16 hours, whereafter most of the volatiles were removed by distillation in vacuo. The residue was partitioned between 10% aqueous sodium hydroxide and methylene chloride.

The organic phase was dried and the solvent removed by distillation. The residue was flash chromatographed on silica gel, eluting with 40% ethyl acetate/60% hexane. The fractions containing the title compound as a free base were combined and the eluant replaced by ether. The ethereal solution was treated with gaseous HCl, the precipitate formed was isolated by suction filtration and recrystallized from isopropanol to yield 7.3g of the title compound, m.p. 182-4°C.

Chromatographic and spectroscopic data were in accordance with U.S. Patent No. 3,513,244, issued May 19, 1970, and an authentic sample, and were as follows: NMR  $\delta$  (CDCl<sub>3</sub>): 2.45 (2H, m), 2.60 (1H, t), 2.90 (1H, m), 3.45 (1H, m), 3.70 (2H, d), 4.95 (1H, t), 7.5 (4H, m) ppm.

**EXAMPLE 2****S-(-)-N-Propargyl-1-aminoindan hydrochloride**

5 The title compound in free base form was isolated by resolving the racemic mixture of the free base of Example 1 on a Chiracel OJ (cellulose tris [p-methylbenzoate]) preparative HPLC column eluting with 10% isopropanol/90% hexane and collecting the first eluted major peak. The  
10 resulting oil was converted to the title compound (hydrochloride) by treatment of a 10% diethyl ether solution of the oil with gaseous HCl, and the resulting precipitate was collected by suction filtration.  $[\alpha]_D -29.2^\circ$  (1%, ethanol), m.p. 182-184°C. Other chromatographic and  
15 spectroscopic properties were identical with the hydrochloride salt of Example 1.

**EXAMPLE 3****R-(+)-N-Propargyl-1-aminoindan hydrochloride**

20 The title compound was prepared as in Example 2 above, except that the second eluted peak from the preparative HPLC was collected:  $[\alpha]_D +29.1^\circ$  (0.8%, ethanol), m.p. 179-181°C. Other chromatographic and spectroscopic properties were  
25 identical with the hydrochloride salt of Example 1.

**EXAMPLE 4****R-(+)-N-Propargyl-1-aminoindan hydrochloride**

30 12.4g of R-(-)-1-Aminoindan and 12.9g of potassium carbonate were added to 95 ml of acetonitrile. The resulting suspension was heated to 60°C and 5.6g of propargyl chloride was added dropwise. The mixture was stirred at 60°C for 16 hours, whereafter most of the volatiles were removed by  
35 distillation in vacuo. the residue was partitioned between



10% aqueous sodium hydroxide and methylene chloride.

The organic phase was dried and the solvent removed in vacuo. The residue was flash chromatographed on silica gel eluting with 40% ethyl acetate/60% hexane. Fractions containing the free base of the title compound were combined and the solvent replaced by ether. The ethereal solution was treated with gaseous HCl and the resulting precipitate was isolated by suction filtration and recrystallized from isopropanol to yield 6.8g of the title compound, m.p. 183-185°C,  $[\alpha]_D^{+30.90}$  (2% ethanol). Spectral properties were identical to those reported for the compound of Example 1.

#### EXAMPLE 5

##### S-(-)-N-Propargyl-1-aminoindan hydrochloride

The title compound was prepared by the method of Example 4, except that S-(+)-1-aminoindan was used as starting material. The product exhibited  $[\alpha]_D^{-30.3}$  (2% ethanol), m.p. 183-5°C. Spectral properties were identical to those reported for the compound of Example 1.

#### EXAMPLE 6A

##### Di(R-(+)-N-propargyl-1-aminoindan) L-tartrate

To a solution of tartaric acid (4.4g) in 48 ml of boiling methanol was added a solution of R-(+)-N-propargyl-1-aminoindan free base (5.0g) in methanol (48 ml). The solution was heated to reflux and 284 ml of t-butylmethyl ether was added over 20 minutes. The mixture was heated for an additional 30 minutes, cooled, and the resulting precipitate was isolated by suction filtration to yield 6.7g of the title compound: m.p. 175-177°C;  $[\alpha]_D$  (1.5, H<sub>2</sub>O) = +34.3; Anal. calcd. for C<sub>28</sub>H<sub>32</sub>O<sub>6</sub>N<sub>2</sub>; C, 68.26, H, 6.56, N, 5.69. Found: C, 68.76; H, 6.57; N, 5.61.

**EXAMPLE 6B****R-(+)-N-propargyl-1-aminoindan mesylate**

5 a) To a solution of propargyl benzenesulfonate (78.4g) and  
racemic aminoindan (63.2g) in toluene (240mL) at 20°C was  
added dropwise an aqueous solution of 15% sodium hydroxide  
(108 mL). After 5 hours of stirring, additional toluene (80  
10 mL) and water (200 mL) were added with stirring. The  
organic phase was separated and washed with 10% aqueous  
sodium hydroxide and then diluted with water. The pH of the  
mixture was adjusted to 3.2 by the addition of 10% aqueous  
sulfuric acid. The aqueous phase was separated and its pH  
was adjusted to 7.3 with 10% sodium hydroxide and extracted  
three times with toluene while maintaining constant pH. The  
15 combined organic layers were concentrated in vacuo to 40.7g  
of a yellow oil.

20 b) The above crude racemic propargylaminoindan and L-  
tartaric acid (10g) were dissolved in isopropanol (1 L) and  
heated to reflux for 1 hour. The reaction was then allowed  
to cool to room temperature with stirring and the  
precipitate collected by filtration. The crude di-  
propargylaminoindan tartrate was recrystallized from 1 L of  
1:1 methanol/isopropanol to give di(R-(+)-N-propargyl-1-  
25 aminoindan)-L-tartrate with physical and spectral properties  
identical to that of the compound of Example 6A.

30 c) A solution of di-(R-(+)-N-propargyl-1-aminoindan)  
tartrate (15g) and methanesulfonic acid (6g) in isopropanol  
(150 mL) was heated to reflux for 30 minutes. The reaction  
was allowed to cool to room temperature and the resulting  
precipitate isolated by suction filtration to give the title  
compound (11.1g) with m.p. 157°C and  $[\alpha]_D = 22^\circ$ .

**EXAMPLE 7****R-(+)-N-Methyl-N-propargyl-1-aminoindan hydrochloride**

5 The free base form of R-(+)-N-propargyl-1-aminoindan from  
Example 4 (1.2 grams), potassium carbonate (0.97 grams) and  
methyl iodide (1 gram) were added to 15 ml of acetone and  
the resulting suspension heated to reflux under a nitrogen  
10 atmosphere for 8 hours. Thereafter the volatiles were  
removed under reduced pressure and the residue partitioned  
between 10% aqueous sodium hydroxide (30 ml) and methylene  
chloride (30 ml). The organic phase was dried and the  
solvent removed in vacuo. The residue was flash  
15 chromatographed on silica gel eluting with 40% ethyl  
acetate/60% hexane. Fractions containing the title compound  
as a free base were combined and the solvent replaced by  
diethyl ether. The ethereal solution was treated with  
gaseous HCl. The volatiles were removed in vacuo, and the  
residue recrystallized from isopropanol to yield 400 mg of  
20 the title compound as a white crystalline solid, m.p. 134-  
136°C,  $[\alpha]_D^{25} +31.40$  (ethanol). NMR  $\delta$  (CDCl<sub>3</sub>): 2.55 (2H, m);  
2.7 (1H, br.s); 2.8 (3H, s); 3.0 (1H, m); 3.4 (1H, m); 3.9  
(2H, br.s); 5.05 (1H, m); 7.7 (4H, m) ppm.

**EXAMPLE 8****S-(-)-N-Methyl-N-propargyl-1-aminoindan hydrochloride**

The title compound was prepared as in Example 7 above,  
except that S-(-)-N-propargyl-1-aminoindan (free base) from  
30 Example 5 was used as the starting material. All of the  
physical and spectral properties of the title compound were  
identical to those in Example 7 except for the  $[\alpha]_D^{25} -34.9^\circ\text{C}$   
(ethanol).

EXAMPLE 9Tablet Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	7.81 mg*
5	Pregelatinized starch NF	47.0 mg
	Lactose NF hydrous	66.0 mg
	Microcrystalline cellulose NF	20.0 mg
	Sodium starch glycolate NF	2.99 mg
	Talc USP	1.5 mg
10	Magnesium stearate NF	0.7 mg
	*Equivalent to 5.0 mg of N-propargyl aminoindan base.	

EXAMPLE 10Tablet Composition

15	N-Propargyl-1(R)-aminoindan Hydrochloride	1.56 mg*
	Lactose hydrous	50.0 mg
	Pregelatinized starch	36.0 mg
	Microcrystalline cellulose	14.0 mg
20	Sodium starch glycolate	2.14 mg
	Talc USP	1.0 mg
	Magnesium stearate NF	0.5 mg
	*Equivalent to 1.0 mg of N-propargyl aminoindan base.	

25 EXAMPLE 11Capsule Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Pregelatinized starch	10.0 mg
30	Starch	44.0 mg
	Microcrystalline cellulose	25.0 mg
	Ethylcellulose	1.0 mg
	Talc	1.5 mg
	Purified water added as required for granulation.	

35

EXAMPLE 12Injection Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
5	Dextrose anhydrous	44.0 mg
	HCl added to pH 5	
	Purified water added as required for 1 ml	

EXAMPLE 1310 Injection Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	1.0 mg
	Sodium chloride	8.9 mg
	HCl added to pH 5	
15	Purified water added as required for 1 ml	

EXAMPLE 14Injection Composition

20	N-Propargyl-1(R)-aminoindan Hydrochloride	2.0 mg
	Sodium chloride	8.9 mg
	HCl added to pH 5	
	Purified water added as required for 1 ml	

25 EXAMPLE 15Syrup Composition

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Sucrose	2250.0 mg
30	Saccharin sodium	5.0 mg
	Methylparaben	6.0 mg
	Propylparaben	1.0 mg
	Flavor	20.0 mg
	Glycerin USP	500 mg
35	Alcohol 95% USP	200 mg

Purified water as required to 5.0 ml

**EXAMPLE 16**

**Sublingual Tablets**

5	N-Propargyl-1(R)-aminoindan Hydrochloride	2.5 mg
	Microcrystalline cellulose	20.0 mg
	Lactose hydrous	5.0 mg
	Pregelatinized starch	3.0 mg
10	Povidone	0.3 mg
	Coloring agent	q.s.
	Flavor	q.s.
	Sweetener	q.s.
	Talc	0.3 mg

15

Blend the excipients and the active and granulate with an ethanol solution of Povidone. After drying and weighing, it is blended with the talc and compressed.

20

**EXAMPLE 17**

**PAI Sublingual Tablets**

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Microcrystalline cellulose	15.0 mg
25	Pregelatinized starch	12.0 mg
	Ethyl cellulose	0.3 mg
	Talc	0.3 mg

Purified water added as required for granulation.

30

**EXAMPLE 18**

**Tablet Composition**

	N-Propargyl-1(R)-aminoindan Hydrochloride	5.0 mg
	Levodopa	100.0 mg
35	Carbidopa	25.0 mg

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	Pregelatinized starch	24.0 mg
	Starch	40.0 mg
	Microcrystalline cellulose	49.5 mg
	Col. D & C Yellow No. 10	0.5 mg
5	Col. D & C Yellow No. 6	0.02 mg
	Alcohol USP added as required for granulation.	

**EXAMPLE 19****Tablet Composition**

10	N-Propargyl-1(R)-aminoindan Mesylate	7.81 mg*
	Pregelatinized starch NF	47.0 mg
	Lactose NF hydrous	66.0 mg
	Microcrystalline cellulose NF	20.0 mg
15	Sodium starch glycolate NF	2.99 mg
	Talc USP	1.5 mg
	Magnesium stearate NF	0.7 mg
	*Equivalent to 5.0 mg of N-propargyl aminoindan base.	

**EXAMPLE 20****Tablet Composition**

	N-Propargyl-1(R)-aminoindan Mesylate	1.56 mg*
	Lactose hydrous	50.0 mg
25	Pregelatinized starch	36.0 mg
	Microcrystalline cellulose	14.0 mg
	Sodium starch glycolate	2.14 mg
	Talc USP	1.0 mg
	Magnesium stearate NF	0.5 mg
30	*Equivalent to 1.0 mg of N-propargyl aminoindan base.	

**EXAMPLE 21****Capsule Composition**

5	N-Propargyl-1(R)-aminoindan Mesylate	5.0 mg
	Pregelatinized starch	10.0 mg
	Starch	44.0 mg
	Microcrystalline cellulose	25.0 mg
	Ethylcellulose	1.0 mg
10	Talc	1.5 mg
	Purified water added as required for granulation.	

The following Examples and the accompanying Tables and Figures relate to biological experiments carried out in accordance with this invention.

**EXAMPLE 22****Inhibition of MAO activity in vitro****Experimental protocol**

The MAO enzyme source was a homogenate of rat brain in 0.3M sucrose, which was centrifuged at 600g for 15 minutes. The supernatant was diluted appropriately in 0.05M phosphate buffer, and pre-incubated with serial dilutions of compounds: R(+)-PAI, S(-)-PAI and racemic PAI for 20 minutes at 37°C. <sup>14</sup>C-Labelled substrates (2-phenylethylamine, hereinafter PEA; 5-hydroxytryptamine, hereinafter 5-HT) were then added, and the incubation continued for a further 20 minutes (PEA), or 30-45 minutes (5-HT). Substrate concentrations used were 50uM (PEA) and 1mM (5-HT). In the case of PEA, enzyme concentration was chosen so that not more than 10% of the substrate was metabolized during the course of the reaction. The reaction was then stopped by addition of tranylcypromine (to a final concentration of 1mM), and the incubate filtered over a



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small column of Amberlite CG-50 buffered to pH 6.3. The column was washed with 1.5ml water, the eluates pooled and the radioactive content determined by liquid scintillation spectrometry. Since the amine substrates are totally retained on the column, radioactivity in the eluate indicates the production of neutral and acidic metabolites formed as a result of MAO activity. Activity of MAO in the sample was expressed as a percentage of control activity in the absence of inhibitors after subtraction of appropriate blank values. The activity determined using PEA as substrate is referred to as MAO-B, and that determined using 5-HT as MAO-A.

### Results

Inhibitory activity of R(+)-PAI, S(-)-PAI and racemic-PAI were examined separately in vitro, and the results of typical experimental runs are shown in Figures 1 and 2. The entire experiment was repeated three times. Concentrations of inhibitor producing 50% inhibition of substrate metabolism (IC-50) were calculated from the inhibition curves, and are shown in Table 1B. From this data it can be seen that:

- (a) the R(+)-PAI is twice as active as the racemate for inhibition of MAO-B;
- (b) the R(+)-PAI is 29 times more active for inhibition of MAO-B than MAO-A;
- (c) the S(-)-PAI is only 1/6,800 as active as the R(+)-PAI for inhibition of MAO-B, and shows little or no selectivity between MAO-B and MAO-A.

Table 1A

5 IC-50 (nM) VALUES FOR INHIBITION OF  
MAO-A AND MAO-B BY RACEMIC-PAI AND THE  
R(+) AND S(-) ENANTIOMERS THEREOF IN  
RAT BRAIN HOMOGENATE IN VITRO

10

<u>IC-50 (nM)</u>					
MAO-A			MAO-B		
S(-)PAI	R(+)PAI	Rac	S(-)PAI	R(+)PAI	Rac
26000	73	140	17000	2.5	5

15

The results of the same experiments using R(+) and S(-) MPAI (N-methyl-N-propargyl-1-aminoindan) are reported in Table 1B. Each of the enantiomers of MPAI is less selective in  
20 MAO-A and MAO-B inhibition than R(+)PAI. Furthermore, R(+)-MPAI is only five times as active as S(-)-MPAI in MAO-B inhibition, in contrast to R(+)-PAI which is about 7000 times as active as S(-)-PAI in this assay.

Table 1B

IC-50 (nM) VALUES FOR INHIBITION OF MAO-A AND MAO-B BY THE  
R(+) AND S(-) ENANTIOMERS OF MPAI IN RAT BRAIN HOMOGENATE  
IN VITRO

5

IC-50 (nM).

	Compound:	<u>MAO-A</u>		<u>MAO-B</u>	
		S(-)MPAI	R(+)MPAI	S(-)MPAI	R(+)MPAI
10		70	3	50	10

Some experiments were also carried out with human cerebral  
cortical tissues obtained 6 hours post-mortem, and treated  
as described above. The results of such an experiment are  
shown in Figure 3, where R(+)-PAI, S(-)-PAI, and racemic PAI  
are as defined herein.

15

EXAMPLE 23

Inhibition of MAO activity in vivo: acute treatment  
Experimental protocol

20

Rats (male Sprague-Dawley-derived) weighing 250±20g were  
treated with one of the enantiomers or the racemic form of  
PAI by intraperitoneal injection (ip) or oral gavage (po)  
and decapitated 1h or 2h later respectively. Groups of  
three rats were used for each dose level of inhibitor, and  
MAO activity determined in brain and liver using the general  
technique described above. The amount of protein in each  
incubation was determined using the Folin-Lowry method, and  
enzyme activity calculated as nmol of substrate metabolized  
per hour of incubation for each mg of protein. Activity of  
MAO in tissues from animals treated with inhibitors was  
expressed as a percentage of the enzyme activity in a group  
of control animals administered vehicle (water for oral

30

35

administration, 0.9% saline for ip injection) and killed as above.

### Results

5 None of the dose levels used with the inhibitor drugs produced any obvious behavioral alteration. The results are depicted in Figures 4 to 11. Following i.p. administration, compound R(+)-PAI produced 90% inhibition of brain MAO-B activity at a dose of 0.5mg/kg. The same dose produced only  
10 20% inhibition of MAO-A activity. By oral administration, the same dose of R(+)-PAI produced 80% inhibition of MAO-B with no detectable inhibition of MAO-A. Essentially similar results were seen for inhibition of hepatic MAO, as for brain MAO. The doses producing 50% inhibition of MAO-A and  
15 MAO-B (IC-50) were calculated from the inhibition curves, and are shown in Table 2. These data show: (a) that MAO inhibitory activity of R(+)-PAI is maintained in vivo in the rat; (b) that selectivity for inhibition of MAO-B, as opposed to MAO-A, by R(+)-PAI is maintained in vivo; (c) that  
20 the much greater activity of the (+)-enantiomer as opposed to the (-)-enantiomer, is maintained in vivo; (d) that the compounds are effectively absorbed after oral administration; and (e) that the compounds effectively pass the blood-brain barrier, and effectively inhibit brain MAO.  
25 The fact that R(+)-PAI was about twice as active as the racemic compound for inhibition of MAO-B is a reflection of the extremely low activity of S(-)-PAI for inhibition of MAO-B.

Table 2

5 IC-50 VALUES (mg/kg) FOR INHIBITION OF MAO-A AND MAO-B  
BY R(+)-PAI, S(-)-PAI OR RACEMIC-PAI, IN THE RAT  
FOLLOWING INTRAPERITONEAL (I.P.) INJECTION OR ORAL  
ADMINISTRATION (P.O.)

IC-50 (mg/kg)							
<u>MAO-A</u>		<u>MAO-B</u>					
10	Compound:	S(-)PAI	R(+)-PAI	Rac	S(-)PAI	R(+)-PAI	Rac
	I.P. BRAIN	>10	1.2	2.5	>10	0.07	0.22
	I.P. LIVER	>10	5	5	>10	0.06	0.11
	P.O. BRAIN	>10	>5	>5	>10	0.17	0.29
	P.O. LIVER	>10	>5	>5	>10	0.05	0.09
15	(Rac = Racemic PAI)						

EXAMPLE 24Inhibition of MAO activity in vivo: chronic treatmentExperimental protocol

20

Rats (specifications as in Example 23, 4 animals for each dose level) were treated with R(+)-PAI or the racemic mixture at three dose levels (0.05, 0.1 and 0.5mg/kg) by oral administration, one dose daily for 21 days, and decapitated 25 2 hours after the last dose. The activities of MAO types A and B were determined in brain and liver as described in Example 23.

Results

30

A daily dose of 0.1mg/kg of compound R(+)-PAI produced a good degree of selective inhibition, with more than 80% inhibition of brain MAO-B and 20% or less inhibition of brain MAO-A. At the higher dose of 0.5mg/kg daily, MAO-A was still inhibited by less than 50% (Figures 12 and 13).  
35 Hepatic MAO showed a similar degree of selective inhibition (Figures 14 and 15). Compound R(+)-PAI was again more potent

than the racemic mixture by a factor of about twofold. In the case of brain MAO, R(+)-PAI had a better degree of selectivity for inhibition of MAO-B than did the racemic mixture.

5

These results show that selectivity of MAO-B inhibition can be maintained following chronic treatment with the compounds. As with other irreversible inhibitors, the degree of enzyme inhibition is greater with chronic treatments than that following a single dose of the drug. Compound R(+)-PAI shows a better degree of selectivity for inhibition of brain MAO-B than the racemic mixture.

10

#### EXAMPLE 25

15

#### Irreversible nature of MAO inhibition

##### Experimental protocol

20

A single dose of compound R(+)-PAI (1 mg/kg) was administered by i.p. injection to groups of 4 rats, and the animals killed 2, 6, 18, 24, 48 and 72 hours later. Activity of MAO-B was determined in whole brain tissues as described hereinabove.

##### Results

25

The results are shown in Figure 16. Maximal inhibition of MAO-B was attained at 6 hours after injection. MAO activity had only returned to 30% of control activity at 72 hours after injection. This experiment demonstrates the irreversible nature of the MAO inhibition by R(+)-PAI.

30

### Results

The results are shown in Table 3. Compound R(+)-PAI at a dose of 1mg/kg (which causes complete inhibition of MAO-B in brain and liver, and 40 to 50% inhibition of MAO-A in these tissues) caused no significant potentiation of tyramine pressor response. At the higher R(+)-PAI dose of 5mg/kg (which causes more extensive inhibition of MAO-A in brain and periphery), there was a significant potentiation of the tyramine pressor response, which was similar in extent to that produced by the same dose of deprenyl, and less than that produced by clorgyline (at a dose which inhibits hepatic MAO-A activity by over 85%).

Table 3

POTENTIATION OF TYRAMINE PRESSOR EFFECT IN CONSCIOUS RATS BY MAO INHIBITORS

Inhibitor	Dose (mg/kg)	No. of rats (n)	Ratio Area Under Pressor Response Curve; After/Before	SEM*
Saline		12	1.25	0.28
Clorgyline	2	6	10.39	2.13
(-)-Deprenyl	1	2	1.15	
(+)-Deprenyl	5	3	2.36	0.16
R(+)-PAI	1	3	1.38	0.7
R(+)-PAI	5	3	3.49	0.98

\*SEM=standard error of the mean

From this experiment it can be concluded that compound R(+)-PAI causes no potentiation of the tyramine pressor effect at a dose which effectively inhibits MAO-B.

EXAMPLE 27Suppression of MPTP-induced dopaminergic toxicity by R(+) PAI

1-Methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that damages nigrostriatal dopaminergic neurons in several mammalian species, including mice, and produces a Parkinsonian syndrome in humans and primates. A crucial initial step in the mechanism of its neurotoxicity involves conversion of MPTP to its toxic metabolite 1-methyl-4-phenyl pyridinium ion (MPP+). This reaction is catalyzed by the enzyme MAO-B and probably takes place outside of dopaminergic neurons, mainly in glia. It is known that MPTP is both a substrate and an irreversible inhibitor of MAO-B. Pretreatment of experimental animals with MAO-B inhibitors such as deprenyl or pargyline protects against and prevents the MPTP-induced damage to nigrostriatal neurons because the oxidative conversion of MPTP to MPP+ is blocked. The progressive nigrostriatal degeneration in Parkinson's may be due to exposure to environmentally-derived exogenous MPTP-like neurotoxins. In such cases, there is an additional strong indication of initiation of sustained treatment with an MAO-B inhibitor from the very early stages of Parkinson's disease in the hope that it will neutralize the damaging effects of such yet putative MPTP-toxins, and thus arrest or slow down the progression of the illness. A successful MAO-B inhibitor drug is currently judged by its ability to block MPTP-induced damage to nigrostriatal dopaminergic neurons in vivo. The (-) and (+) enantiomers of PAI were therefore tested for their potency in preventing or attenuating the MPTP-induced striatal dopamine depletions in mice.

Experimental Protocol

Male C57 black mice (20-25g weight) were (a) injected with MPTP·HCl (30 mg/kg dissolved in distilled water, s.c.), or vehicle alone, or one hour after pretreatment with the (-)



or (+) isomers of PAI (2.5 mg/kg, i.p.), or with deprenyl (5 mg/kg, i.p.), and (b) decapitated 5 days later. Brains were removed and corpora striata dissected on an ice-cold glass plate and frozen on dry ice. Striatal tissues were homogenized in 0.1 M perchloric acid, and deproteinized aliquots containing dihydroxybenzylamine as an internal standard were assayed for dopamine and its major metabolite 3,4-dihydroxy-phenylacetic acid (DOPAC) using HPLC with electrochemical detection.

### Results

Table 4 shows the results of this experiment. Treatment with MPTP alone produced marked striatal dopamine (DA) and DOPAC depletions. Treatment with the (-) and (+) enantiomers of PAI or with (-) deprenyl did not affect striatal DA concentrations. Pretreatment with the (-) isomer of PAI did not affect the MPTP-induced DA and DOPAC levels in the striatum. The (+)-isomer of PAI given before MPTP completely abolished the reduction in striatal DA and DOPAC levels produced by the toxin. At a dose of 2.5 mg/kg, (+)PAI was equipotent to (-) deprenyl (5 mg/kg) in its protective effect.

Table 4

EFFECT OF PRETREATMENT WITH THE (-) AND (+) ENANTIOMERS OF  
THE MAO-B INHIBITOR PAI ON THE STRIATAL DA AND DOPAC  
DEPLETIONS INDUCED BY MPTP IN MICE IN VIVO

		<u>DA</u>	<u>DOPAC</u>
		(ng/mg protein)	
	Control	162.8±7.2	8.4±0.5
	MPTP	53.1±6.2	3.2±0.3
5			
10	(-)PAI	174.0±4.8	7.5±0.2
	(-)PAI + MPTP	53.4±6.9	7.0±0.6
	(+)PAI	185.0±6.9	3.3±0.3
	(+)PAI + MPTP	177.8±14.4	6.0±0.3
	(-)Deprenyl	170.6±7.1	5.6±0.3
15	(-)Deprenyl + MPTP	197.0±8.0	6.4±0.5

Above values for DA and DOPAC expressed as Mean±S.E.M. and number of rats. n = 7-11 in each group.

20 These results indicate that the R(+)PAI is an excellent MAO-B inhibitor in vivo, and is of especially great potential for the treatment of Parkinson's disease.

25 While the invention has been described with reference to the aforementioned Examples and the accompanying Tables and Figures, it is not restricted thereto. Various modifications and applications of the invention are possible. For example, (R)-PAI may be combined, in a synergistic way, with  $\alpha$ -tocopherol (a vitamin E derivative)  
30 for the treatment of Parkinson's disease.

EXAMPLE 28Effect of PAI enantiomers on amphetamine induced stereotype behavior in senescent rats

5 Amphetamine is known to induce stereotypic behavior (Sulser, F., and Sanders-Bush, E., Ann. Rev. Pharmacol., 11, 209-230 (1971)) by the mobilization of endogenous dopamine. Amphetamine is not metabolized by MAO-B. Inhibition of MAO-B by an effective inhibitor and administration of  
10 amphetamine cause release of dopamine which will not undergo degradation by the inhibited MAO-B. Thus, an increase of synaptic dopamine is expected after administration of amphetamine and effective MAO-B inhibitor leading to an  
15 increase in stereotype behavior-potentiation of the amphetamine effect. The extent of this behavior is rated in accordance with the number of lateral head movements over a period of 1 minute.

Experimental Protocol

20 The test compound was administered at a dose of 0.5 mg/kg/day in drinking water, 24 hours before the infliction of hypoxia (92% nitrogen + 8% oxygen for 6 hours). Following that, amphetamine was injected s.c. at a dose of 0.5 mg/kg. 45 minutes later, lateral head movements were  
25 counted.

Results

The results of these experiments are shown in Table 5.

Table 5

EFFECT OF PAI ISOMERS ON AMPHETAMINE-INDUCED STEREOTYPE  
BEHAVIOR IN SENESCENT RATS (CONTROL AND HYPOXIA LESIONED)

5					
	<u>Group</u>			<u>Stereotype</u>	
				<u>Treatment Behavior Rating</u>	
	Control (6)	-		87±10	
	Control (5)	(+) PAI		126±16*	
10	Control (4)	(-) PAI		94±18	
	Hypoxia lesioned (5)	-		93±12	
	Hypoxia lesioned (6)	(+) PAI		143±6*	
15	Numbers in parentheses are numbers of animals tested. *P<0.001 with respect to untreated hypoxia group or untreated control group correspondingly.				
20	The results in Table 5 indicate that (+)PAI caused significant potentiation of the amphetamine-induced stereotype behavior in both hypoxia-lesioned and control rats. (-)PAI was totally inactive in this respect. These behavioral <u>in vivo</u> results corroborate previous biochemical				
25	findings that (+)PAI is an active inhibitor of MAO-B in the brain while (-)PAI is inactive in this respect.				

EXAMPLE 29Effect on R(+)-PAI on the improvement or restoration of memory.

5 Newborn rat pups subjected to a brief episode of anoxia and then allowed to resume their growth in a normal way develop a long-lasting impairment of memory (Speiser, et al., Behav. Brain Res., 30, 89-94 (1988)). This memory impairment is expressed as an inferior performance in the passive  
10 avoidance test.

The effect of R(+)-PAI and S(-)-PAI on the improvement or restoration of memory was investigated in the passive avoidance test. If the drug is effective, it increases the  
15 latency of response to enter a dark compartment or chamber where an electroshock has been experienced earlier by the rat being tested. The latency of the maximal response is 300 seconds.

20 Experimental Protocol

Young rats were subjected to post-natal anoxia as described in Example 27. R(+)-PAI or S(-)-PAI were administered according to one of the following protocols.

25 Protocol A - Nursing mothers were given a dose of either isomer of 1-1.5 mg/kg/day in drinking water until weaning at 21 days. Following that, the weaned offsprings were directly treated with the same dose for 20 days. Treatment was terminated at 40 days and the test was performed at 60  
30 days, that is 20 days after the last dose of the drug.

Protocol B - The dose was reduced to 0.5 mg/kg/day administered to the nursing mother until weaning at 21 days, then directly to the young rats to 60 days at which time the  
35 test was performed.

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Passive Avoidance Test - The apparatus consisted of a lit chamber adjoining a dark chamber and a sliding door separating the two. At training, a rat was placed in the lit chamber for 30 seconds, and then the door was opened. The rat moved to the dark chamber with a latency that was recorded. Upon entry of the rat into the dark compartment, the door was closed and a 0.3 mA foot-shock was delivered for 3 seconds.

Retention (memory) after 48 hours was determined by repeating the test and recording the latency to step through from light to darkness to an arbitrary maximum of 300 seconds.

### Results

The results of these experiments are shown in Table 6.

Table 6

EFFECT OF PAI ISOMERS ON PASSIVE AVOIDANCE  
RESPONSE IN YOUNG RATS (60-DAYS OLD)

#### PROTOCOL A

	<u>Group</u>	<u>Treatment</u>	<u>Before</u> <u>Electroshock</u>	<u>After</u> <u>Electroshock</u>
25	Control	-	49 ± 13	201 ± 111
	Control	(+) PAI	49 ± 19	220 ± 100 (+9%)*
	Control	(-) PAI	48 ± 13	192 ± 116
	Anoxia-lesioned	-	45 ± 11	183 ± 109
30	Anoxia-lesioned	(+) PAI	49 ± 10	239 ± 99 (19%)*
	Anoxia-lesioned	(-) PAI	55 ± 27	179 ± 123

PROTOCOL B

	<u>Group</u>	<u>Treatment</u>	<u>Before</u> <u>Electroshock</u>	<u>After</u> <u>Electroshock</u>
	Control	-	53 ± 20	104 ± 101
5	Control	(+) PAI	48 ± 11	128 ± 119 (+23%)*
	Anoxia-lesioned	-	45 ± 8	119 ± 105
	Anoxia-lesioned	(+) PAI	52 ± 12	137 ± 126 (+15%)*
	Anoxia-lesioned	(-) PAI	48 ± 19	112 ± 112

10

Figures represent the latency in seconds for entering a dark compartment where an electroshock had been first experienced by the rat tested.

15

\* The indicated percent increases are with respect to the corresponding anoxia or control groups.

20

The experimental results indicated that (+)PAI but not (-) PAI is effective in improving the memory of anoxia-lesioned and control rats. Drugs active in this test are considered to be potentially useful for treatment of various memory impairment disorders, dementia and especially senile dementia of the Alzheimer's type.

25

EXAMPLE 30Effect of R(+)-PAI on the anoxia-induced hyperactive syndrome in juvenile rats

30

Rats that had been exposed postnatally to anoxia and then left to grow under normal conditions show increased motor activity in the open field at the age of 10-42 days (Hertshkowitz, et al., Dev. Brain Res., 7, 145-155 (1983)).

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The effect of R(+)-PAI and S(-)-PAI on such hyperactive syndrome was investigated.

#### Experimental Protocol

5 Anoxia was performed on rat pups on the first post-natal day. They were placed in a glass chamber and exposed to 100% nitrogen for 25 minutes. They were resuscitated by intermittent massage softly applied to the chest and then returned to their respective mothers. Control rats received  
10 the same treatment but with air instead of nitrogen.

The R(+)-PAI or S(-)-PAI (0.5 mg/kg/day) was administered to the nursing mothers in drinking water, thereby transferred to the sucklings through milk.

15 Locomotion was measured in 6 fully computerized cages (28 x 28 cm) by recording the number of crossings over a given period of time. Crossings of grid/infrared beams at 4-cm intervals initiated electrical impulses which fed a counter.  
20 Recordings of motor activity were made at the ages of 15 and 20 days, over a period of 15 minutes.

#### Results

25 The experimental results are given in Table 7.



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Table 7EFFECT OF EACH OF THE TWO ENANTIOMERS ON THE  
ANOXIA-INDUCED HYPERACTIVE SYNDROME

5	<u>Group</u>	<u>Treatment</u>	15-day old <u>rats</u>	20-day old <u>rats</u>
	Control	-	414 ± 192 (11)	808 ± 212 (12)
	Control	(+) PAI	254 ± 149 (11) c	719 ± 110 (13)
10	Anoxia- lesioned	-	482 ± 119 (7)	858 ± 96 (9)
	Anoxia- lesioned	(+) PAI	276 ± 186 (15) a	737 ± 150 (16) c
15	Anoxia- lesioned	(-) PAI	334 ± 196 (5)	778 ± 232 (6)

Numbers in parenthesis are numbers of animals tested.  
- The figures are the numbers of crossings of infrared beam grid in the activity cage over a period of 15 minutes.

20

- a P<0.001 compared to anoxia untreated group.  
b P<0.05 compared to anoxia untreated group.  
c P<0.05 compared to control group.

25

These results indicate that chronic oral treatment with R(+)-PAI at a dose of 0.5 mg/kg administered to the nursing mother and reaching the milk-fed offspring significantly improved the hyperactive syndrome. Consequently, R(+)-PAI is a potentially useful drug for the treatment of the hyperactive syndrome in children.

30

EXAMPLE 31Stability differences among ten salts of PAI

35

Stability is an important factor in the selection of an optimal salt as a therapeutic drug. Different salts may alter the physicochemical and biological characteristics of a drug and can have a dramatic influence on its overall

properties. (Berge, S.M., et al., J. Pharm. Sci. 66, 1 (1977); Gould, P.L., Int. J. Pharmaceutics, 33, 201 (1986)).

### Experimental

#### 5 Synthesis of PAI salts

A solution of an appropriate acid (1 mol-eq.) in 2-propanol was added to a solution of PAI (1 mol-eq.) while stirring in 2-propanol (Ar, BHT). The salt formed was filtered, washed with 2-propanol and ether, and dried under low pressure.  
10 Yields were between 70 to 90%. An exception in preparing PAI acetate involved using ether as the solvent.

#### Analytical methods

The chromatographic separations were carried out using a  
15 Lichrosphere 60 RP select B 5 $\mu$  125x4 mm (Merck) column, an HPLC (Jasco BIP-1) equipped with a L-4200 UV-Vis detector (Merck-Hitachi) set to 210 nm, and a D-2500 chromatointegrator (Merck-Hitachi). The eluent and diluent consisted of 80% distilled water/20% acetonitrile (HPLC  
20 grade), and 0.07 M perchloric acid adjusted to pH 2.5 with aqueous ammonia. The flow rate used was 1 ml/min, the appropriate PAI salt solution concentration was 250  $\mu$ g/ml, and 20  $\mu$ l of the solution were injected onto the chromatographic system.

25 The melting range was measured with an automatic apparatus (Mettler FP 80) and thermo-gravimetric analysis was performed on a Mettler TA 3000 system at a rate of 10°C/min in the applicable range. Solubility was determined by an  
30 appropriate dilution of the supernatant from a saturated PAI salt water solution and measured in a UVIKON 941 (Kontron) UV-Vis spectrophotometer. The salt form (mono- or di-salt) was obtained by elemental analysis using standard equipment for C, H, N and S determination. The pH was measured in a  
35 1% aqueous solution of the PAI salts.

Results

The characterization of the various salts are summarized in Table 8.

5

Table 8

## PHYSICOCHEMICAL PROPERTIES OF PAI SALTS

	PAI-salt m.w.	pH	Solubility mg/ml	Melting range (°C)	%Wt. loss	Salt form
10	tartarate 492	5.5	33	176.2-177.3	LT 0.1	di
15	mesylate 267	4.3	635	156.8-157.6	0.1	mono
	maleate 287	4.0	NLT 1000	87.2-87.8	0.1	mono
20	sulphate 440	3.9	485	159.4-161.1	3.2	di
	chloride 207	4.2	238	177.0-180.0	LT 0.5	mono
	tosylate 343	4.4	60-70	129.3-129.9	LT 0.1	mono
25	fumarate 287	3.5	95	125.4-126.2	0.2	mono
	phosphate n.a.	7.0	NLT 720	109.5-110.4	n.a.	n.a.
30	esylate 279	2.4	NLT 300	n.a.	n.a.	mono
	acetate 231	6.1	NLT 720	69.2-69.7	0.4	mono

35 n.a.= not available

40 Comparative stability studies were carried out under sets of several accelerating conditions: I) heating at 80°C for 72, 96 or 144 hours; and II) reflux in isopropanol for 30 hours. The degradation products developed were measured by HPLC and confirmed by TLC. The results are presented in Table 9 with the relative retention time (relative to the

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PAI peak; RRT) as an area percentage relative to total integrated peak area.

Table 9

5

DEGRADATION PRODUCTS DEVELOPED IN PAI SALTS UNDER SHORT TERM CONDITIONS

10	Salt	80C/72h		80C/144h		Reflux in	
		RRT <sup>a</sup>	% <sup>b</sup>	RRT	%	iPrOH/30h	
	sulfate	ND <sup>c</sup>	ND	ND	ND	RRT	%
						0.47	0.22
						0.60	0.72
15	phosphate	0.60	0.22	0.60	0.57	0.60	2.62
						0.74	0.21
						1.84	0.20
						1.98	0.73
20	chloride	ND	ND	ND	ND	2.23	0.71
	mesylate	ND	ND	ND	ND	0.60	0.08
	maleate	0.60	0.41	n.a.		0.60	2.17
25		1.27	0.50			0.65	1.35
		1.48	0.33			1.29	0.59
		1.81	0.10			1.42	1.30
		3.07	1.44			1.50	0.16
		4.16	0.10			1.83	0.18
30		4.84	7.76			1.98	0.23
						4.09	0.65
	acetate	0.44	0.10	n.a.		0.60	6.74
		0.60	2.56			0.74	0.35
35		0.73	0.13			1.76	0.33
		1.29	0.71			1.84	0.16
		1.55	1.06			1.99	4.17
		1.75	21.85			3.60	0.27
		1.96	3.33				
40		2.15	0.08				
		2.32	0.15				
		2.83	0.15				
		3.54	1.82				
45	esylate <sup>d</sup>	ND	ND	0.85	0.26	ND	ND
				1.96	0.31		

limit of quantitation=0.08%

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n.a.=not available

<sup>a</sup>Relative retention time (relative to the PAI peak).<sup>b</sup>Area percentage relative to total integrated peak area.<sup>c</sup>No impurities detected.<sup>d</sup>Ethyl sulfate salt.

The salts were submitted to visual inspection of color and form. The findings are shown in Table 10.

Table 10

## APPEARANCE OF PAI SALTS UNDER DESTRUCTIVE CONDITIONS

Salt	80°C/72h	80°C/96h	80°C/144h	reflux in iPrOH/30h
sulfate	off white powder	n.a.	off white powder	brown powder
phosphate	brownish powder	n.a.	brown powder	brown powder
chloride	white powder	n.a.	white powder	off white powder
mesylate	white powder	n.a.	white powder	white powder
maleate	brown melted	brown	n.a.	brown melted
esylate	brownish melted	n.a.	dark brown melted	dark brown melted

n.a.= not available

These studies show that sulphate, esylate and mesylate possess significant advantages relative to the other salts due to good solubility and chemical stability. Of these three salts, mesylate is preferable due to its excellent stability even under destructive conditions.

EXAMPLE 32Reversal of haloperidol-induced catalepsy in mice

Male, ICR mice 25-30g each, were pretreated with either of  
5 the following drugs: Saline, (R)-PAI mesylate, or racemic-  
PAI mesylate. All drugs were administered i.p. in a volume  
of 0.2 mL. Two hours later, haloperidol was injected s.c.  
at a dose of 6mg/kg in a volume of 0.1-0.2 mL. Motor  
10 coordination tests were made at 3 hours after giving  
haloperidol, that is, 5 hours after administering the  
presumed protective drugs.

Motor coordination tests and rigidity were quantified  
according to three different parameters: (a) ability to  
15 walk the length of a horizontal rod, 80 cm-long; (b) ability  
to climb down, face down, a vertical rod, 80 cm-long; (c)  
duration of immobility in an unnatural sitting posture  
whereby the abdomen of the mouse is pressed against a  
"wall." Full performance as in haloperidol-untreated mice  
20 is given the score of 4 in each test, or a total of 12 in  
all tests. Poor performance is given a score from 1 to 3.  
A key to score ratings is given in Table 9A. The effects of  
the various agents in antagonizing haloperidol-induced  
catalepsy are given in Table 11. At three hours after  
25 haloperidol, (R)-PAI mesylate conferred protection against  
haloperidol at 5-15 mg/kg, reaching a peak after effect at  
7.5-10 mg/kg (activity score  $\approx$  94% of saline control).  
Racemic PAI mesylate conferred partial protection in the  
range of 7.5-15 mg/kg, and was not active at 5 mg/kg. From  
30 Figure 17, it can be seen that the dose-effect profile of  
either (R)-PAI mesylate or racemic PAI is such that an  
increase in dose beyond 10 mg/kg entails a decrease in  
effect, but that the racemic mixture is less potent  
throughout. This means that racemic PAI mesylate at twice

the dose of (R)-PAI mesylate will always be less active than the (R) enantiomer.

Reversal of  $\alpha$ -MPT-induced hypokinesia in rats

5 The drug  $\alpha$ -MPT is assumed to inhibit the formation of L-DOPA from tyrosine, and consequently the formation of dopamine itself. Lack of CNS dopamine is expressed as hypoactivity. Six month-old male Wistar rats (from Harlan Orkack, UK) were pretreated with saline, (R)-PAI Mesylate or Rac PAI  
10 Mesylate, at the indicated doses. Two hours later they received i.p.  $\alpha$ -MPT at a dose of 100 mg/kg in 0.3-0.5 mL. Controls received saline. Following this, motor activity was recorded in a computerized activity cage for the duration of 10 hours. The results are given in Table 12 and  
15 Figure 18. At 2 mg/kg, (R)-PAI Mesylate restored the level of activity to about 90% of the saline-treated rats, but Rac PAI Mesylate was not active. In either case, the profile of the dose-effect curve was bell-shaped, suggesting a decrease in effect with an increase in dose beyond a peak of 2-5  
20 mg/kg. At 5 mg/kg Rac PAI Mesylate could not elicit a level of activity comparable to that of (R)-PAI Mesylate at 2 mg/kg.

From these measurements, (R)-PAI Mesylate and Rac PAI  
25 Mesylate do not share a similar pattern of activity in the restoration of normokinesia in haloperidol-treated mice and  $\alpha$ -MPT-treated rats. At all doses studied, (R)-PAI Mesylate is always more potent than Rac PAI Mesylate at the corresponding dose. Also, peak activity of Rac PAI Mesylate  
30 is always lower than peak activity of (R)-PAI Mesylate. Thus, Rac PAI Mesylate at a given dose is always less effective than (R)-PAI Mesylate at half the same dose. Doubling the dose of Rac PAI Mesylate with respect to (R)-PAI Mesylate does not produce an effect equivalent to that  
35 of (R)-PAI Mesylate.

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Pharmacologically, Rac PAI Mesylate cannot be considered as consisting of 50% active ingredient which is (R)-PAI Mesylate and 50% inert material as diluent. The presence of (S)-PAI in Rac PAI Mesylate has an adverse effect on the activity of (R)-PAI, resulting in a more than two-fold decrease in potency. The decrease may be due to a direct adverse effect of (S)-PAI on behavioral parameters.

Table 11

REVERSAL OF HALOPERIDOL-INDUCED CATALEPSY IN MICE  
WITH (R)-PAI MESYLATE AND RACEMIC MESYLATE

Mice received each of the test drugs i.p. at the indicated doses. Two hours later they received haloperidol as described in the text. The doses shown are for the free base.

	Dose, mg/kg	(R)-PAI Mesylate			Rac PAI Mesylate		
		<u>Score+SE</u>	<u>n</u>	<u>% of control</u>	<u>Score+SE</u>	<u>n</u>	<u>% of control</u>
20	1.8	7.2±1	6	60	7.0±0.6	6	59
	3.0	6.4±0.5	6	60	5.9±0.7	6	49
	5.0	8.7±0.9*	6	73	6.4±0.4	6	53
	7.5	11.0±0.4***	5	92	9.4±0.8++	6	78
25	10	11.3±0.3***	6	94	9.2±0.6***	6	77
	15	10.8±0.5***	5	90	8.8±0.8*	6	73
30	Control saline	12±0	12	100			
	Haloperidol alone	6.6±0.3	16	59			

Statistical significance with respect to haloperidol alone: \* $p \leq 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$  by the Student's "t" test. The scores for (R)-PAI are significantly different from those of racemic PAI at 5 mg/kg,  $p \leq 0.05$ ; at 10 mg/kg,  $p \leq 0.01$ ; and at 15 mg/kg,  $p \leq 0.05$ .



Table 11A

5 KEY TO SCORE RATING OF HALOPERIDOL-INDUCED CATALEPSY  
IN MICE AND ITS REVERSAL BY VARIOUS AGENTS

Vertical Rod:

	Unable to grasp rod with limbs	1
	Able to grasp but slips down	2
10	Able to grasp, partly slips, partly climbs down	3
	Able to grasp, climbs down using all limbs	4

Horizontal Rod:

	Unable to grasp, falls off rod	1
15	Able to grasp, unable to walk on rod more than 2 paces	2
	Able to grasp, walks half-length of rod	3
	Able to grasp, walks full length of rod	4

Immobility Sitting Against Wall:

20	Immobility > 5 min	1
	Immobility 3-5 min	2
	Immobility 1-3 min	3
	Immobility 0.1 min	4

25 Fractional scores are assigned, such as 2.5, when behavior falls between two categories, as between 2 and 3.

Table 12

RESTORATION OF MOTOR ACTIVITY IN RATS TREATED WITH  
 $\alpha$ -METHYL-p-TYROSINE ( $\alpha$ -Mpt) AT 100 mg/kg i.p.

Rats received the test drugs i.p. at the indicated doses. After two hours they received  $\alpha$ -Mpt and were immediately placed in activity cages. Total motor activity was automatically recorded for 10 hours, as described in the text.

Dose, mg/kg	(R) -PAI Mesylate			Rac PAI Mesylate		
	<u>Score+SE</u>	<u>n</u>	<u>% of control</u>	<u>Score+SE</u>	<u>n</u>	<u>% of control</u>
2	14,132** $\pm 1457$	7	89	9,035 $\pm 829$	6	57
5	12,893* $\pm 1,869$	7	81	10,926* $\pm 820$	8	69
7.5	6,679 $\pm 414$	4	42	9,698 $\pm 557$	4	61
Control saline	15,862 $\pm 1,424$	5	100			
$\alpha$ -Mpt alone	8,108*** $\pm 810$	6	51			

Statistical significance by the Student's "t", \* $p \leq 0.01$ ;  
 \*\*\* $p \leq 0.001$  for Test drugs+  $\alpha$ -Mpt versus  $\alpha$ -Mpt alone  
 $\alpha$ -Mpt alone versus control saline  
 The scores of (R)-PAI versus racemic PAI are significantly  
 different at 2 mg/kg,  $p \leq 0.01$ .

EXAMPLE 33The effects of (R)-PAI Mesylate following closed head injury in rats5     Methods1.     Induction of trauma

Head trauma was induced in male rats under ether anesthesia by a well calibrated weight-drop device that falls over the exposed skull, covering the left cerebral hemisphere, 1-2 mm lateral to the midline, in the midcoronal plane.

2.     Evaluation of motor function

One hour after induction of trauma, the rats were tested by a set of criteria which evaluated their neurologic outcome (the criteria described by Shohami, et al., J. Neurotrauma, 10, 113 (1993)). These criteria, referred to as the Neurological Severity Score (NSS), consist of a series of reflexes and motor functions. Points are given based on deficits in these criteria. At 24h the rats were re-evaluated.

3.     Evaluation of brain edema

The brains were removed after the second evaluation of motor function (24h). A piece of tissue (~20 mg) was weighed to yield wet weight (WW). After drying in a desiccator oven for 24h at 95°C, it was reweighed to yield dry weight (DW). Water percentage in the tissue was calculated as  $(WW - DW) \times 100 / WW$ .

30    4.     Drug treatment

(R)-PAI Mesylate was dissolved in water. The rats were injected intraperitoneally at a dose of 0.1 mg/kg, 0, 4, 8 and 12 h after induction of head trauma. Control rats were treated with water at the same times.

35

Results

The NSS, which measures the "clinical" status of the rats, was almost identical in the treated and nontreated groups at 1 hour after the head injury, but significantly lower at 24 hours in the (R)-PAI mesylate-treated rats (Table 13). These results indicate that PAI mesylate is effective in improving motor function recovery following closed head injury in rats.

At 24 hours after trauma, a major edema was found in the hemisphere (85.4% water in the brain of control rats vs. 78.5% in undamaged brain tissue). PAI mesylate was effective in reducing edema as verified by its effect on the percent of water.

In conclusion, the results reported herein demonstrate that (R)-PAI mesylate has neuroprotective properties in a model intended to mimic human nerve injury and to induce trauma to a closed skull.

Table 13

	NSS		$\Delta$ NSS (1h - 24h)	% H <sub>2</sub> O in the brain
	1 h	24 h		
control (n = 6)	15.6	12.3	4.3 $\pm$ 0.5	85.4 $\pm$ 0.4
(R)-PAI Mesylate (n = 6)	16.7	10.2	6.5 $\pm$ 0.7*	82.1 $\pm$ 0.6**

\* P < 0.05 (Mann Whitney U-test)

\*\* P < 0.005 (t-test)

EXAMPLE 34Effects of PAI mesylate on prevention of NMDA induced cell death of cerebellum cell culturesResults of in vitro assays

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Procedures: Cultures of mechanically dissociated neonatal rat cerebellum. The cerebella are dissected aseptically from 6 or 7-day-old rat pups and placed in a 15 ml sterile plastic conic tube containing 3 ml of enriched medium (the medium is made up of Dulbecco's modified Eagle's medium (DMEM) with high glucose concentration (1g/l), 2 mM (v/v) L-glutamine, antibiotic antimetabolic mixture, and enriched with 15% (v/v) heat-inactivated fetal calf serum). The cerebella are then dissociated after 20-25 passages through a sterile 13 gauge, 10 cm long stainless steel needle attached to a 5 ml syringe with an inserted 45  $\mu$ m pore size nylon sieve. The dissociated cells are centrifuged at 200g for 5 minutes, the supernatant discarded and the cells resuspended in enriched medium. The cell viability is determined by the trypan blue exclusion test. The cells are then plated at a density of 200/mm<sup>2</sup> on poly-L-lysine-coated surfaces (Poly-L-lysine-coated glass coverslips are prepared at least 1 hour before plating, by immersing in a sterile distilled water solution containing 15  $\mu$ g/ml poly-L-lysine, and just before use, washing with sterile water and drying), covered with enriched medium, and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> in air and 100% humidity. After 4 days in culture, the media are replaced with media containing the desired test compounds. Experiments are done in duplicate and repeated 2 or 3 times. After determining the test compound toxic dose-response, four groups are compared: (I) control (enriched medium alone), (II) test compound (one subgroup for each concentration (2 concentrations are tested)), (III) N-methyl-D-aspartate (NMDA, exposure to a concentration of 1 mM for 3 h) as the cytotoxic challenge, (IV) test compound

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plus NMDA (one subgroup for each of the 2 concentrations of test compounds), (V) control group to test the effect of solvent (in which the test compound is dissolved), and (VI) an additional "positive control" group of spermine (0.01  $\mu$ M dissolved in culture medium) plus NMDA. Nerve cell survival is evaluated by phase contrast microscopy and trypan blue staining after 24 h.

### Results

It is well established that glutamic acid (Glu) possesses neurotoxic properties which are expressed in several neurological disorders including epilepsy and stroke, and most likely also in brain neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and traumatic brain injury. The neurotoxic effects of Glu are mediated by membrane bound Glu receptors, such as N-methyl-D-aspartate (NMDA) receptors.

The results, as shown in Table 14, demonstrate that 10  $\mu$ M of (R)-PAI mesylate increased the survival of cerebellum cells by 27 percent following 1  $\mu$ M NMDA exposure. These in vitro results support the in vivo effects of (R)-PAI mesylate presented in Examples 33 and 35, indicating that this drug has neuroprotective properties against neurotoxic concentration of NMDA.

Table 14

5                    NEUROPROTECTIVE EFFECT OF (R)-PAI MESYLATE ON  
PREVENTION OF NMDA-INDUCED CELL DEATH OF CEREBELLUM  
CELLS

	Experimental Group	Surviving Cells	Percent Protection
10	<u>Cerebellar Cultures</u>		
	(Toxicity $TD_{25} = 30 \mu M$ ; $TD_{50} = 85 \mu M$ ; $TD_{100} = 320 \mu M$ )		
	Control	100	
15	Solvent	97	
	NMDA	10	
	Solvent + NMDA	10	0
	Compound + NMDA:		
	1) 0.01 $\mu M$ + "	12	2
20	2) 1.00 $\mu M$ + "	22	12
	3) 10.00 $\mu M$ + "	37	27
	Spermine + NMDA	75	65

25                    Values, expressed as the percent of untreated controls,  
represent the average of 2 experiments run in duplicate for  
culture experiments, and the mean  $\pm$  SEM of 4 animals for  
ischemia. The percent protection value is the effect of the  
test compound after subtraction of the solvent effect.

EXAMPLE 35Effects of (R)-PAI Mesylate after graded crush of the rat optic nerve

5      Neuroprotective effects of (R)-PAI Mesylate were determined for application immediately after crush injury of the optic nerve in the adult rat. Short-term effects were measured metabolically, and long-term effects electrophysiologically.

10      METHODS

1.      Metabolic measurements

- 15      a) General. The method is described by Yoles, et al., Investigative Ophthalmology & Visual Science, 33, 3586-91 (1992). At short terms, metabolic measurements were monitored in terms of the mitochondrial NADH/NAD ratio, which depends on the activity of the electron transport system, and thus indicate levels of energy production.
- 20      Changes in ability of the nerve to produce energy as a consequence of injury were determined by comparing NADH levels in response to artificial transient anoxic insult before and after the injury.
- 25      b) Surface fluorometry - reflectometry. Monitoring of the intramitochondrial NADH redox state is based on the fact that NADH, unlike the oxidized form NAD<sup>+</sup>, fluoresces when illuminated at 450 nm. A flexible Y-shaped bundle of optic fibers (light guide) was used to transmit the light to and
- 30      from the optic nerve. The light emitted from the nerve was measured at two wavelengths: 366 nm (reflected light) and 450 nm (fluorescent light). Changes in the reflected light were correlated with changes in tissue absorption caused by hemodynamic effects and with movements of the optic nerve
- 35      secondary to alterations in arterial blood pressure and



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nerve volume. The fluorescence measurements were found to be adequately corrected for NADH redox state measurements by subtraction of the reflected light (366 nm) from the fluorescent light (1:1 ratio) to obtain the corrected fluorescence signal.

c) Animal preparation. Animal utilization was in accord with the ARVO Resolution on the use of animals in research. Male Sprague-Dawley (SPD) rats weighing 300-400g were anesthetized with sodium pentobarbitone (50 mg/kg intraperitoneally). With the animal's head held in place by a head holder, a lateral canthotomy was performed under a binocular operating microscope and the conjunctiva was incised lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was identified and a length of 3-3.5 mm was exposed near the eyeball by blunt dissection. The dura was left intact and care was taken not to injure the nerve. A special light-guide holder was implanted around the optic nerve in such a way that the light guide was located on the surface of the optic nerve 1 mm distal to the injury site. Animals, while still anesthetized, were allowed to recover for 30 minutes from the surgical procedures and were then exposed to anoxic conditions. An anoxic state was achieved by having the rat breathe in an atmosphere of 100% nitrogen for 2 minutes, after which time it was returned to air. In order to evaluate the metabolic activity of the optic nerve, the relative changes in reflected and fluorescent light intensities in response to anoxia were measured before and after crush injury.

d) Experimental protocol for crush injury and metabolic measurements. With the aid of calibrated cross-section forceps, a moderate crush injury was inflicted on the nerve between the eye and the light guide holder at a pressure

corresponding to 120g for 30 sec. Immediately after injury, animals received intraperitoneal injections of water with and without (R)-PAI Mesylate (2 mg/kg). To assess the activity of the energy production system, NADH response to 2 minutes of anoxia was measured in all animals prior to injury, 30 minutes after injury, and thereafter at hourly intervals up to 4 hours (see Figure 19).

2. Electrophysiological Measurements. This method is described by Assia, et al., Brain Res., 476, 205-212 (1989). Animal preparation and optic nerve injury were preferred as in the metabolic studies. Immediately after injury, animals received a single injection of water with or without (R)-PAI Mesylate (0.5 mg/kg). Fourteen days after injury and treatment, the optic nerves were excised and measured electrophysiologically. Prior to removal of optic nerves for electrophysiological measurement, the rats were deeply anesthetized with 70 mg/kg pentobarbitone. The skin was removed from the skull and the optic nerves were detached from the eyeballs. Subtotal decapitation was performed and the skull was opened with a rongeur. The cerebrum was displaced laterally, exposing the intracranial portion of the optic nerve. Dissection was at the level of the nerve, which was transferred to vials containing fresh salt solution consisting of NaCl (126 mM), KCl (3 mM),  $\text{NaH}_2\text{PO}_4$  (1.25 mM),  $\text{NaHCO}_3$  (26 mM),  $\text{MgSO}_4$  (2mM),  $\text{CaCl}_2$  (2 mM), and D-glucose (10 mM), and aerated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  at room temperature. The nerves were kept in this solution, in which electrical activity remained stable for at least 3-4 hours. After 0.5 hours of recovery at room temperature, electrophysiological recordings were obtained from the nerve distal to the crush lesion. The nerve ends were then connected to two suction Ag-AgCl electrodes immersed in a bathing solution at 37°C. A stimulating pulse was applied through the electrode at the proximal end and the action

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potential was recorded by the distal electrode. A Grass SD9 stimulator was used for supramaximal electrical stimulation (0.5 pps). The measured signal was transmitted to a Medelec PA36 preamplifier and then to an electromyograph (Medelec MS7, AA7T amplifier). The solution, stimulator and amplifier had a common ground. The maximum amplitude of eight averaged compound action potentials (CAPs) was recorded and photographed with a Polaroid camera. The CAP values measured in contralateral uninjured nerves served as a reference.

### Results

The results demonstrate that (R)-PAI Mesylate applied immediately after optic nerve injury blocked the injury-induced reduction in energy production. (R)-PAI Mesylate also has a long-term effect measured by electrophysiological monitoring.

The CAP (compound action potentials) amplitude is directly correlated with the number of conducting fibers in the tested segment of the nerve.

(R)-PAI Mesylate significantly attenuated the injury-induced loss of activity in the distal segment of the injured nerve, indicating that (R)-PAI Mesylate is a neuroprotective agent or at least slows down degeneration.

Table 15  
Electrophysiological Measurements

Group	CAP amplitude ( $\mu$ V) (Mean $\pm$ Std. Error.)
Vehicle N = 13	441 $\pm$ 95
(R)-PAI Mesylate N = 7	2104 $\pm$ 313*

EXAMPLE 36

Comparison of Anticonvulsive Properties of R-PAI and S-PAI Salts

Both (R)-PAI and (S)-PAI HCl salts have significant anticonvulsant activities. In mice (i.p. administration) in the maximal electroshock test (MES test), (S)-PAI HCl has greater anticonvulsant activity ( $ED_{50}$ =57 mg/kg) than (R)-PAI HCl ( $ED_{50}$ =79 mg/kg). Analogous results were observed in rats (p.o. administration). Four out of four rats were protected from seizures in the MES test when 50 mg/kg of (S)-PAI HCl was administered, whereas three out of four mice were protected after the same dose of (R)-PAI HCl. With respect to efficacy for Parkinson's disease, the enhanced anticonvulsant activity is a detrimental side effect. The same trend occurs with the mesylate salts. (S)-PAI Mesylate has greater anticonvulsant activity than (R)-PAI Mesylate in the MES test. At doses of 100 mg/kg, (S)-PAI Mesylate protected three out of three mice, whereas only one out of three mice was protected with (R)-PAI Mesylate.

The MES test is a classical model to indicate efficacy for partial and generalized seizures in humans. The agents' mechanism of action is via their ability to prevent the spread of seizures. Some agents, however, that prevent seizure spread have the side effect of lowering seizure threshold. These agents therefore have both proconvulsive and anticonvulsive side effects.

Results herein show that (S)-PAI Mesylate has proconvulsive activity. In the Timed Intravenous Infusion of Metrazol test, 141 mg/kg of (S)-PAI Mesylate reduces the time, and therefore the amount of Metrazol, required to induce the appearance of both the first focal seizure and the onset of clonus. Other agents that are classically used for partial and generalized seizures, such as phenytoin and carbamazepine, do not show this effect. (H.J. Kupferberg, *Epilepsia*, 30, s51-s56 (1989)). Likewise, (S)-PAI Mesylate showed a significantly higher acute neurotoxicity than (R)-PAI Mesylate. At 300 mg/kg, (R)-PAI Mesylate did not show any neurotoxicity with mice in the rotorod ataxia test. With (S)-PAI Mesylate, four out of four mice showed neurotoxicity and spasticity.

#### Methods

TD<sub>50</sub> (median toxic dose). This test measures neurological deficits by the rotorod ataxia test. A mouse is placed on a knurled rod rotating at 6 rpm. It is then determined whether a mouse has the ability to maintain its equilibrium and stay on the rod for one minute in each of three trials.

Timed Intravenous Infusion of Metrazol Test. This test measures the minimal seizure threshold of each animal. Metrazol is infused at 0.185 mg/ml into the tail veins of mice. The time is then recorded (sec) from the start of infusion until the appearance of the first twitch (first

focal seizure) and onset of clonus (clonic seizure). Proconvulsants require less Metrazol to produce these symptoms and therefore show endpoints at a shorter period of time.

5

EXAMPLE 37

Peripheral effects of (R)-PAI and (S)-PAI on the contractility of intestinal smooth muscle preparations

10 Peripheral effects of the hydrochloride salts of the enantiomers of PAI were determined in isolated rabbit or guinea-pig small intestine. These observations provide useful information on their relative peripheral side effects in humans. The first point of contact of the subject with  
15 an orally administered drug is the gastrointestinal tract where concentrations of the drug are much higher than after absorption and distribution. In the case of PAI hydrochloride (MW = 208), a 10 mg oral dose contained in a liquid volume of about 100 ml would be equivalent to a  
20 concentration of about 0.5 mM. In contrast, the therapeutic plasma concentration of (R)-PAI hydrochloride is in the nanomolar range.

The effect of the enantiomers of PAI in the isolated rabbit  
25 jejunum and the guinea-pig ileum were determined so as to find out whether the intake of (S)-PAI together with (R)-PAI (as found in racemic PAI) would produce side effects absent in the administration of pure (R)-PAI. (R)-PAI is the preferred enantiomer for the inhibition of MAO-B in the  
30 brain, in view of its potency and high selectivity towards this form of the enzyme. (S)-PAI is much less potent than (R)-PAI in this respect and is also not selective toward MAO-B. In principle, its presence in PAI racemate might be tolerated or overlooked provided (S)-PAI is inert at the  
35 recommended doses of (R)-PAI. The results provided in

Tables 16-19 show that (S)-PAI is not an inert substance. On the contrary, in the guinea-pig ileum, it is a more potent relaxant than (R)-PAI. Hence its peripheral effects cannot be discounted as negligible. These data show that there would be fewer peripheral side effects in the administration of pure (R)-PAI than in the administration of racemic PAI containing an equivalent dose of (R)-PAI.

Table 16

TYRAMINE POTENTIATION BY EACH OF THE TWO ENANTIOMERS  
OF PAI HCl IN RATE JEJUNUM PREPARATION

A stretch of rabbit jejunum, mounted in an organ bath, displays rhythmic contractions that are inhibited by norepinephrine but not by tyramine. If however the jejunum is pretreated with a monoamine oxidase inhibitor such as PAI, then tyramine causes relaxation of the spontaneous contractions. The extent of relaxation can be correlated with the relative potency of the inhibitor.

<u>Drug and concentration (<math>\mu</math>M)</u>		<u>P e r c e n t</u> <u>relaxation</u>
Tyramine alone	40	0
Norepiniphrine	0.002	100
(R)PAI alone	0.2-4.0	0
(S)PAI alone	0.2-4.0	0
Tyramine	40	
after (R)PAI	0.2	67
	2	88
	40	85-90
after (S)PAI	0.2	0
	2	35
	40	33-50

Results

(S)-PAI is much less potent than (R)-PAI as an inhibitor of brain MAO-B. Therefore, (S)-PAI is not a useful agent for the prevention of brain dopamine degradation, but can potentiate the tyramine-evoked release of norepinephrine in the small intestine. Its activity in the small intestine is an undesirable side effect as it is expected to increase the absorption and action of undegraded tyramine. Thus, (S)-PAI is not an inert substance when used together with (R)-PAI as found in racemic PAI.

Table 17

ANTAGONISM OF BETHANECHOL-INDUCED CONTRACTIONS OF THE GUINEA PIG ILEUM PREPARATION IN THE PRESENCE OF 400  $\mu$ M OF EACH OF THE TWO ENANTIOMERS OF PAI HCl

A stretch of guinea-pig ileum mounted in a physiological solution in an organ bath contracts dose-dependently when treated with bethanechol which is an enzymatically stable analog of the natural gastrointestinal neurotransmitter acetylcholine. These contractions are attenuated in the presence of PAI. The data are expressed in gram-tension.

	<u>Bathenechol (<math>\mu</math>M)</u>	<u>gram-tension</u>			
		<u>control</u>	<u>(R) PAI</u>	<u>control</u>	<u>(S) PAI</u>
	0.8	0.5	0.2	0.6	0
	2	1.5	0.3	2.0	0
	4	2.2	0.7	3.0	0
30	8	4.0	1.0	3.8	0.6
	20	5.6	2.0	3.8	1.2
	40	6.2	2.8	3.8	1.7
	80	6.2	3.1	3.8	2.6
	200	6.2	4.3	3.8	2.6

35



Results

(S)-PAI is almost inactive as a MAO-B inhibitor with respect to (R)-PAI, and hence is not effective in preventing the degradation of brain dopamine. However, it is more effective than R(PAI) in the prevention of the bethanechol-induced contraction of the small intestine. Thus (S)-PAI is not an inert substance when used with R(PAI) as found in racemic PAI.

Table 18

ANTAGONISM OF THE HISTAMINE-INDUCED CONTRACTIONS OF THE  
GUINEA-PIG ILEUM PREPARATION BY EACH OF THE TWO  
ENANTIOMERS OF PAI HCl

5

A fixed dose of histamine (40 nM) causes a sustained contraction of a stretch of guinea-pig ileum mounted in physiological solution in an organ bath. Incremental addition of each of the two enantiomers of PAI HCl causes a dose-dependent relaxation of the muscle. Results are expressed as percent relaxation with respect to the base-line before addition of histamine, which is taken as 100% relaxation.

PAI concentration	Percent relaxation	
	<u><math>\mu</math>M</u>	<u>(R) PAI</u> <u>(S) PAI</u>
	2	0      11
	4	0      15
	10	0      30
20	20	20
	30	31      33
	40	37      36
	100	81      71
	200	90
25	300	92
	400	100      98
	700	100
	1000	100

30

Results

(S)-PAI is inactive with respect to (R)-PAI as a MAO-B inhibitor in the brain, and hence useless for preventing the degradation of brain dopamine, but is more active than the (R) isomer in causing relaxation of intestinal smooth

muscle. Thus, (S)-PAI is not an inert substance when taken together with the (R) isomer as found in racemic PAI.

Table 19

5           ANTAGONISM OF THE BETHANECHOL-INDUCED CONTRACTIONS OF  
            THE GUINEA-PIG ILEUM PREPARATION BY EACH OF THE TWO  
                                                    ENANTIOMERS OF PAI HCl

10           A fixed dose of bethanechol (0.8  $\mu$ M) causes a sustained  
            contraction of a stretch of guinea-pig ileum mounted in  
            physiological solution in an organ bath. Incremental  
            addition of each of the two enantiomers of PAI HCl causes a  
            dose-dependent relaxation of the preparation. Results are  
15           expressed as percent relaxation with respect to the base-  
            line before addition of histamine, which is taken as 100%  
            relaxation.

20	PAI concentration	Percent relaxation	
	<u><math>\mu</math>M</u>	<u>(R) PAI</u>	<u>(S) PAI</u>
	20	25	40-50
	60	25-50	60-70
	100	50-70	100
	300	100	100

25

Results

30           (S)-PAI is inactive with respect to (R)-PAI as a MAO-B  
            inhibitor in the brain, and hence useless for the prevention  
            of the degradation of brain dopamine, but is more active  
            than the (R) isomer in causing relaxation of intestinal  
            smooth muscle. Thus, (S)-PAI is not an inert substance when  
            taken together with the (R) isomer as found in racemic PAI.

EXAMPLE 38**SOME EFFECTS OF [R] (+) PAI MESYLATE IN MIDDLE CEREBRAL ARTERY  
OCCLUSION IN THE RAT AS A MODEL FOR STROKE**

5

Methods

1.1. *Middle cerebral artery occlusion (MCAO) in the rat.*  
A modification of the procedure described by Tamura et al  
10 (1981) was used. Male Wistar rats (Olac England-Jerusalem)  
300-400 g each were anesthetized with a solution of  
Equitesine administered i.p. at a dose of 3 mL/kg.  
Equitesine consists of 13.5 mL sodium pentothal solution (60  
15 mg/mL), 3.5 g chloral hydrate, 1.75 g MgSO<sub>4</sub>, 33 mL propylene  
glycol, 8.3 mL absolute alcohol made up to 83 mL with  
distilled water. Surgery was performed with the use of a  
high magnification operating microscope, model SMZ-2B, type  
102 (Nikon, Japan). In order to expose the left middle  
20 cerebral artery, a cut was made in the temporal muscle. The  
tip of the coronoid process of mandible was excised as well  
and removed with a fine rongeur. Craniectomy was made with  
a dental drill at the junction between the median wall and  
the roof of the inferotemporal fossa. The dura matter was  
opened carefully using a 27 gauge needle. The MCA was  
25 permanently occluded by microbipolar coagulation at low  
power setting, beginning 2-3 mm medial to the olfactory  
tract between its cortical branch to the rhinal cortex and  
the laterate striate arteries.

30 After coagulation, the MCA was severed with microscissors  
and divided to ensure complete occlusion. Following this,  
the temporalis muscle was sutured and laid over the  
craniectomy site. The skin was closed with a running 3-0  
silk suture. A sham craniectomy operation was performed on  
35 a parallel group of rats, but without cauterization of the

MCA. During the entire surgical operation (20-25 min) in either group, body temperature was maintained at 37 to 38 °C by means of a body-temperature regulator (Kyoritsu, Japan) consisting of a self-regulating heating pad connected to a rectal thermistor. At 24 hours post surgery a neurological score was taken in order to assess the severity of the injury in the drug-treated rats with respect to their untreated controls. At 48 hours, the animals were anesthetized with Equitesine and the severity of the injury was visualized by an MRI procedure. The volume of brain tissue incurring damage following ischemia was determined.

### 1.2. Drug administration

[R] (+)PAI Mesylate was administered as an i.p. injection in 0.3-0.4 mL distilled water, according to the following schedule:

1 mg/kg immediately after surgery.

0.5 mg/kg 2 hours after surgery

1 mg/kg 20-24 hours after surgery

### 1.3. MRI scan of ischaemic brain lesion

All experiments were performed using a 4.7T BIOSPEC system (BRUKER) (See T. Back, et al., "Diffusion Nuclear Magnetic Resonance Imaging in Experimental Stroke: Correlation with Cerebral Metabolites," Stroke (February 1994) 25: 494-500). Forty-eight hours after MCAO or sham operation, every animal was subjected to a fast multislices T1 weighted imaging (TR/TE), (500/25) for positioning. Then multislices T2-weighted images (3000/80) were acquired (5 contiguous slices, 3 mm thick).

The size and severity of the infarcted area was estimated using the hyperintensity observed in the T2 weighted MRI at 48 hours post-occlusion or post sham-operation. The

following MRI parameters were determined for each group of rats:

- c. Ischemic area (in mm<sup>2</sup>)
- 5 d. Area of the ischemic hemisphere (in mm<sup>2</sup>)
- e. Area of the unaffected hemisphere (in mm<sup>2</sup>)

10 The use of contiguous slices allows the conversion of area units into volume units by simply multiplying the area value by the slice thickness.

#### 1.4. Neurological score

15 The neurological score consists of the sum total of a series of ratings assigned to the performance of specific locomotor activities in a given rat. The scale runs from 0 (fully normal rats) to 13 (fully incapacitated rats). Most parameters are rated as either 0 (normal), or 1 (incapacitated); others are graded. The following tests were used in the present study:

20

General observational tests: hypoactivity; sedation; piloerection

25

Motor reflex. Rats were lifted by the tail about 15 cm above the floor. Normal rats assume a posture in which they extend both forelimbs towards the floor and spread the hind limbs to the sides in a trapeze-like manner. MCAO when severe causes consistent flexion of the contralateral limb.

30

Motor ability. This is seen as the ability to grasp a rod 1 cm in diameter by the contralateral limb for 5-15 sec when the rat is left hanging on the rod through the arm pit.

35

Motor coordination. Normal rats are able to walk up and down a beam 5 cm wide placed at a moderate slant. Failure

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to walk the beam in either direction reveals some motor incoordination, lack of balance and limb weakness.

5     Gait.    Ability to restore normal position to either hind contralateral limb when intentionally displaced while on a narrow beam.

10     Balance.   Ability to grasp and balance on a narrow beam 2 cm wide.

10     Locomotor activity.   Total movements over a period of 15 min in an automated activity cage.

15     Ratings assigned to each of the above parameters are given in Table 20.

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Table 20. Neurological scores assigned to each of 10 parameters of posture and locomotion

	<u>Parameter</u>	<u>Score</u>
5	a. Activity in the home cage	normal=0
		hypoactive=1
10	b. Sedation	none=0
		marked=1
15	c. Piloerection	none=0
		marked=1
20	d. Extension of contralateral forelimb towards floor when lifted by tail	good=0
		flexed limb=1
25	e. Spread of contralateral hind limb when lifted by tail (trapezoid posture)	good=0
		flexed limb=1
30	f. Grasp rod with contralateral limb for 5-15 sec. when suspended by the armpit	good=0      poor=1
35	g. Walk on beam 5-cm wide	good=0      poor=1
30	h. Restoration of contralateral hind and or fore limb to original position when intentionally displaced	good=0
		poor=1 (one limb) 2 (two limbs)
35	i. Grasping and balance on beam 2-cm wide	good=0      poor=1



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j. Motor activity with respect to control (for 15 min in an automated activity cage		≤25% of control	3
		26-50% of control	2
5		51-75% of control	1
		76-100% of control	0

## 2. Results

### 10 2.1. Infarct size

The results of the MRI study are summarized in Table 21 and Figure 20. The infarct size was significantly smaller in [R](+)PAI Mesylate-treated rats (n=9) than in untreated rats (n=10). In the former, the infarct size was about 60% of that  
15 in the untreated animals.

TABLE 21

Ischaemic brain lesion evaluation by MRI T2-SCAN - 48hrs following MCA-Occlusion and [R] (+)PAI Mesylate treatment in 5 Wistar Rats.

MCA-O		MCA-O + [R] (+)PAI Mesylate*	
Animal No.	Infarct size (mm <sup>3</sup> )	Animal No.	Infarct size (mm <sup>3</sup> )
1	252	1	94.4
2	272	2	139
3	314	3	240
4	273	4	137
5	201	5	137
6	221	6	174
7	358	7	164
8	265	8	171
9	341	9	215
10	236		
MEAN $\pm$ SD	273.3 $\pm$ 50.9	MEAN $\pm$ SD	163.5 $\pm$ 43.9

$$t = 5.0475$$

$$f = 17$$

$$p < 0.001$$

[R] (+)PAI Mesylate reduces infarct size by 40% significantly

- \* [R] (+)PAI Mesylate administered:  
 Time after MCA-Occlusion:  
 0 -1.0mg/kg ip;  
 2hrs -0.5mg/kg ip;  
 24hrs -1.0mg/kg ip.

## 2.2. *Neurological score*

The neurological score in five [R](+)PAI Mesylate treated rats and six untreated rats were determined by a blinded observer. The results are given in Table 22 where they are compared with the infarct size in each animal as determined by the MRI test, and also in Fig. 21. It can be seen that those animals with the least neurological scores were those treated with [R](+)PAI Mesylate. The neurological score was reduced by 54% and the infarct size by 36% in [R](+)PAI Mesylate-treated MCAO rats as compared to untreated ones.

TABLE 22

Neurological score of Rats submitted to MCA-Occlusion and  
[R](+)PAI Mesylate treatment with relation to their  
5 ischaemic infarct size.

MCA-O			MCA-O + [R](+)PAI Mesylate***		
Animal No.	Neurological * Score	Infarct size** (mm <sup>3</sup> )	Animal No.	Neurological * Score	Infarct size** (mm <sup>3</sup> )
1	5.0	201	1	1.0	137
2	5.0	221	2	2.0	174
3	6.0	358	3	4.0	164
4	6.0	265	4	4.0	171
5	8.25	341	5	2.88	215
6	5.75	236			
MEAN + SD	6.0±1.19	270±65	MEAN +, SD	2.78±1.3	172±28

Neurological Score

Infarct Size

t = 4.25

t = 3.34

f = 9

f = 9

p &lt; 0.01

p &lt; 0.01

[R](+)PAI Mesylate reduces the neurological score by 53.7% and infarct size by 36.3%.

\* Examined 24hrs after MCA-Occlusion.

\*\* Evaluated by MRI T2-SCAN 48hrs after MCA-Occlusion.

\*\*\* [R](+)PAI Mesylate administered:

Time after MCA-Occlusion:

0 -1.0mg/kg ip;

2hrs -0.5mg/kg ip;

24hrs -1.0mg/kg ip.

## References for Example 38

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What is claimed is:

- 5           1.    A method of treating brain ischemia or stroke in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat brain ischemia or stroke in the subject.
- 10          2.    The method of claim 1, wherein the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of:  
the mesylate salt;  
the ethylsulfonate salt;  
15          the sulfate salt; and  
the hydrochloride salt.
- 20          3.    The method of claim 2, wherein the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.
- 25          4.    The method of claim 1, wherein the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of the subject.
- 30          5.    The method of claim 1, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered intravenously, orally, rectally, transdermally, or parenterally.
- 35          6.    The method of claim 1, wherein the subject is human and the effective amount is from about 0.01 mg to 50.0 mg per day.
7.          7.    The method of claim 6, wherein the effective amount is from 0.1 to 10.0 mg per day.

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8. The method of claim 6, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered intravenously.
- 5 9. The method of claim 1, wherein the area of the brain ischemia is reduced by about thirty-five percent.
- 10 10. A method of treating head trauma injury in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat head trauma injury in the subject.
- 15 11. The method of claim 10, wherein the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of:  
the mesylate salt;  
the ethylsulfonate salt;  
the sulfate salt; and  
20 the hydrochloride salt.
- 25 12. The method of claim 11, wherein the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.
- 30 13. The method of claim 10, wherein the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of the subject.
- 35 14. The method of claim 10, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered orally, rectally, transdermally, or parenterally.



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15. The method of claim 10, wherein the subject is human and the effective amount is from about 0.01 mg to 50.0 mg per day.
- 5 16. The method of claim 15, wherein the effective amount is from 0.1 to 10.0 mg per day.
- 10 17. The method of claim 15, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered intravenously.
- 15 18. A method of treating spinal trauma injury in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat spinal trauma injury in the subject.
- 20 19. The method of claim 18, wherein the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of:  
the mesylate salt;  
the ethylsulfonate salt;  
the sulfate salt; and  
the hydrochloride salt.
- 25 20. The method of claim 19, wherein the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.
- 30 21. The method of claim 18, wherein the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of the subject.
- 35 22. The method of claim 18, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof

is administered orally, rectally, transdermally, or parenterally.

- 5           23. The method of claim 18, wherein the subject is human and the effective amount is from about 0.01 mg to 50.0 mg per day.
- 10           24. The method of claim 23, wherein the effective amount is from 0.1 to 10.0 mg per day.
25. The method of claim 23, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered intravenously.
- 15           26. A method of treating neurotrauma in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat neurotrauma in the subject.
- 20           27. The method of claim 26, wherein the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan is selected from the group consisting of:  
            the mesylate salt;  
25           the ethylsulfonate salt;  
            the sulfate salt; and  
            the hydrochloride salt.
- 30           28. The method of claim 27, wherein the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.
- 35           29. The method of claim 26, wherein the effective amount is from about 0.5 milligrams per kilogram body weight of the subject to about 2.5 milligrams per kilogram body weight of the subject.

30. The method of claim 26, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered orally, rectally, transdermally, or parenterally.
- 5 31. The method of claim 26, wherein the subject is human and the effective amount is from about 0.01 mg to 50.0 mg per day.
- 10 32. The method of claim 31, wherein the effective amount is from 0.1 to 10.0 mg per day.
- 15 33. The method of claim 31, wherein the R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is administered intravenously.
- 20 34. A method of treating a subject afflicted with a neurodegenerative disease which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat the neurodegenerative disease in the subject.
- 25 35. A method of treating a subject afflicted with a neurotoxic injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat the neurotoxic injury in the subject.
- 30 36. A method of treating a subject afflicted with brain ischemia which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat brain ischemia in the subject.
- 35 37. A method of treating a subject afflicted with a head trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or

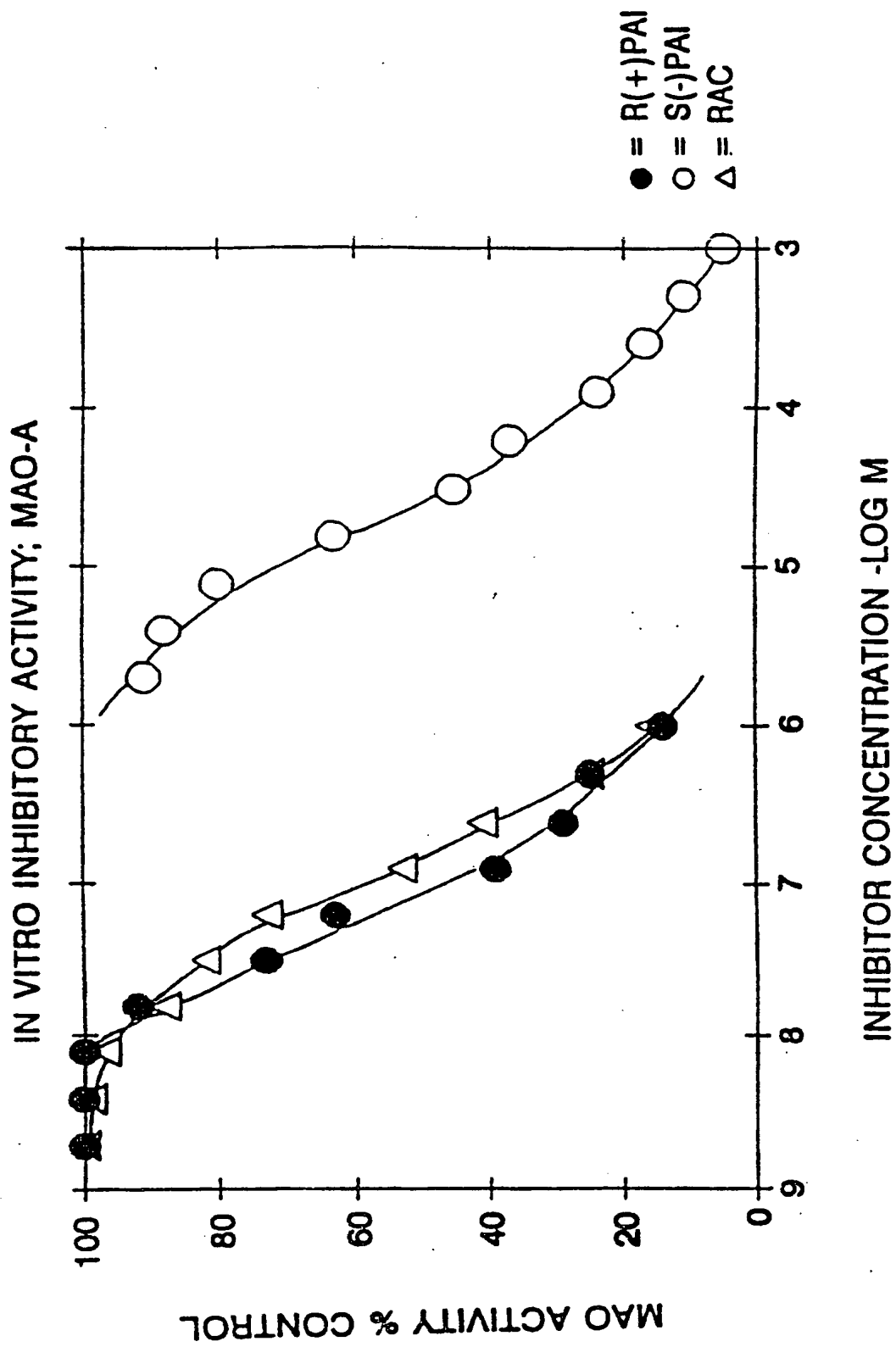
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a pharmaceutically acceptable salt thereof effective to treat the head trauma injury in the subject.

- 5           38. A method of treating a subject afflicted with a spinal trauma injury which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to treat the spinal trauma injury in the subject.
- 10          39. A method of preventing nerve damage in a subject which comprises administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or a pharmaceutically acceptable salt thereof effective to prevent nerve damage in the subject.
- 15          40. The method of claim 39, wherein the nerve damage is structural nerve damage.
- 20          41. The method of claim 39, wherein the structural nerve damage is optic nerve damage.

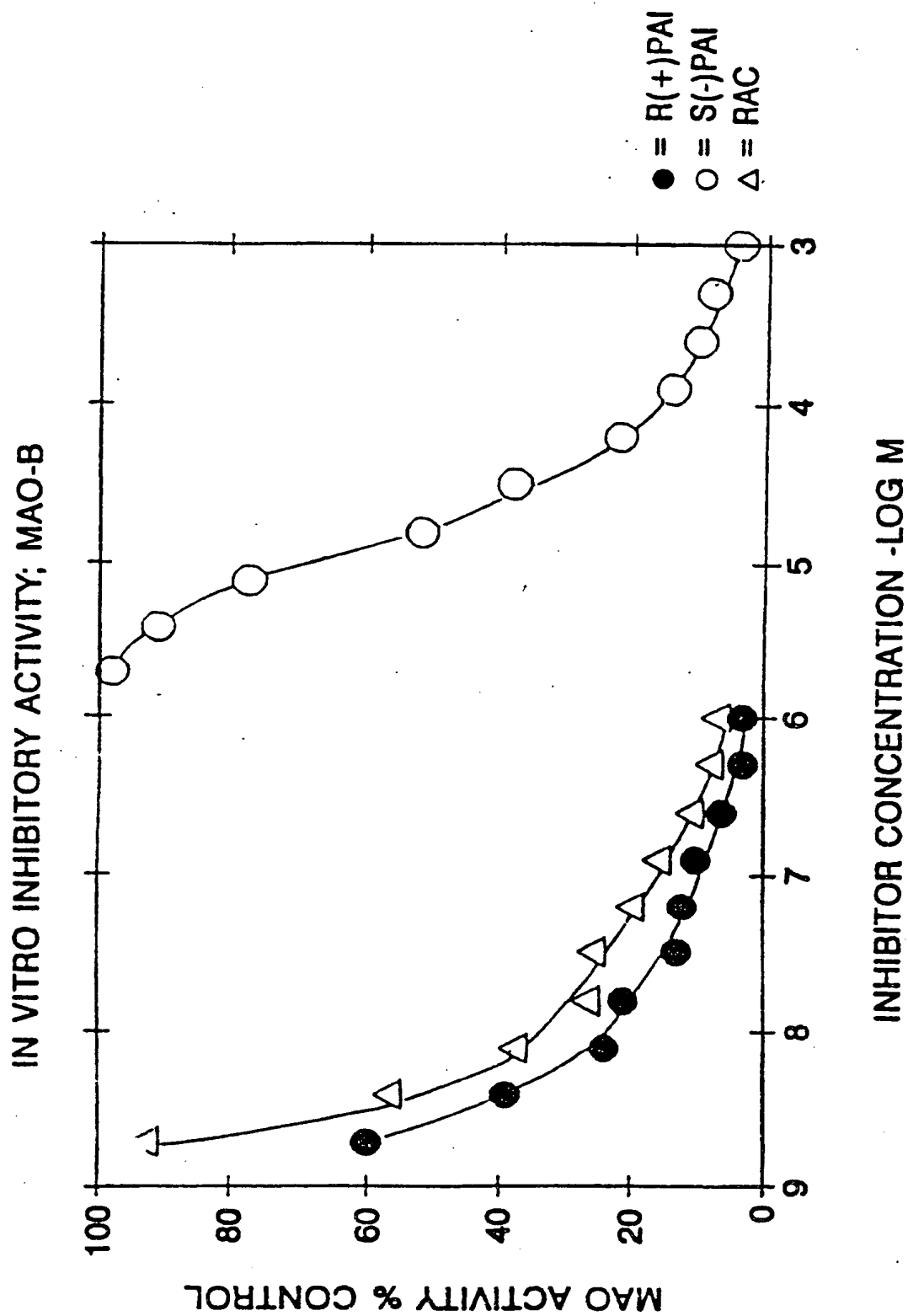
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FIGURE 1



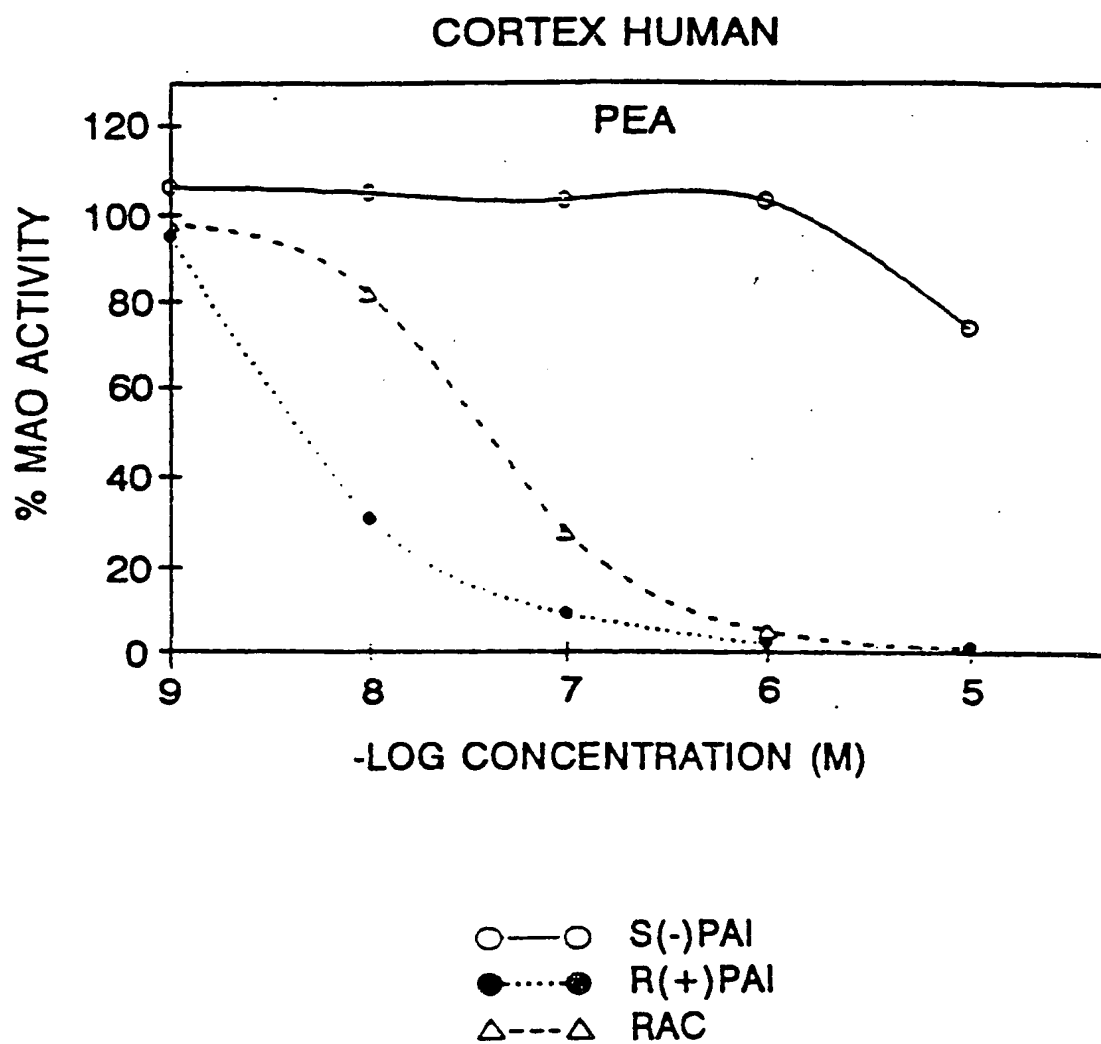
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FIGURE 2



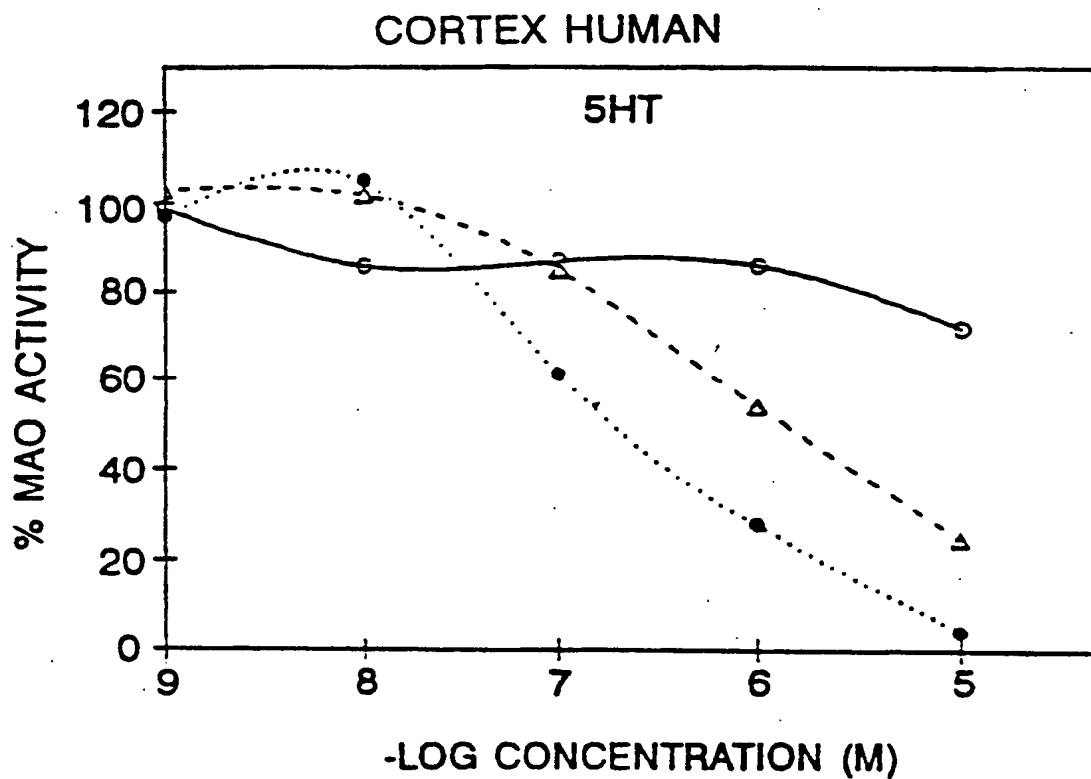
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FIGURE 3A



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FIGURE 3B

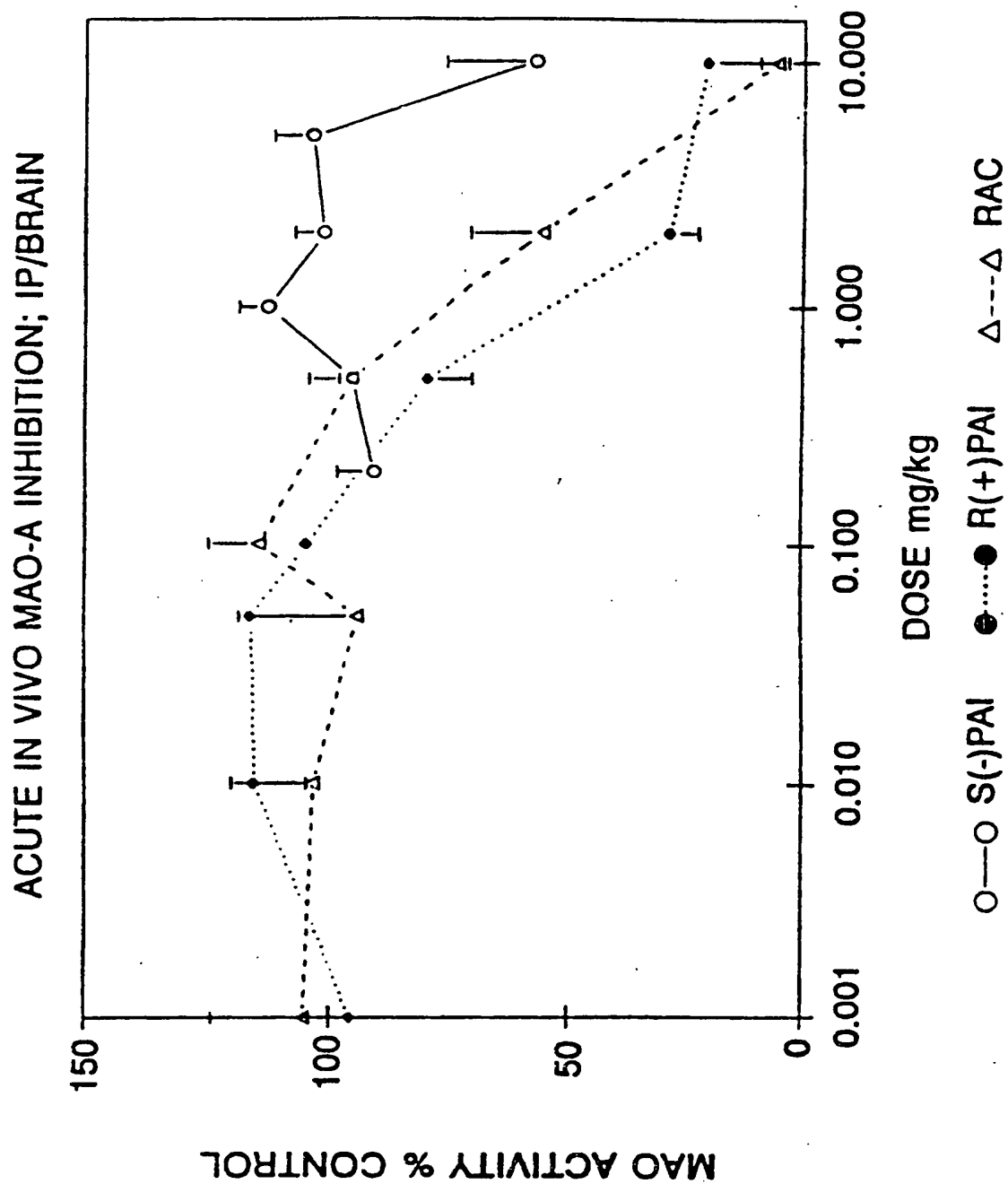


○—○ S(-)PAI  
●·····● R(+)-PAI  
△---△ RAC



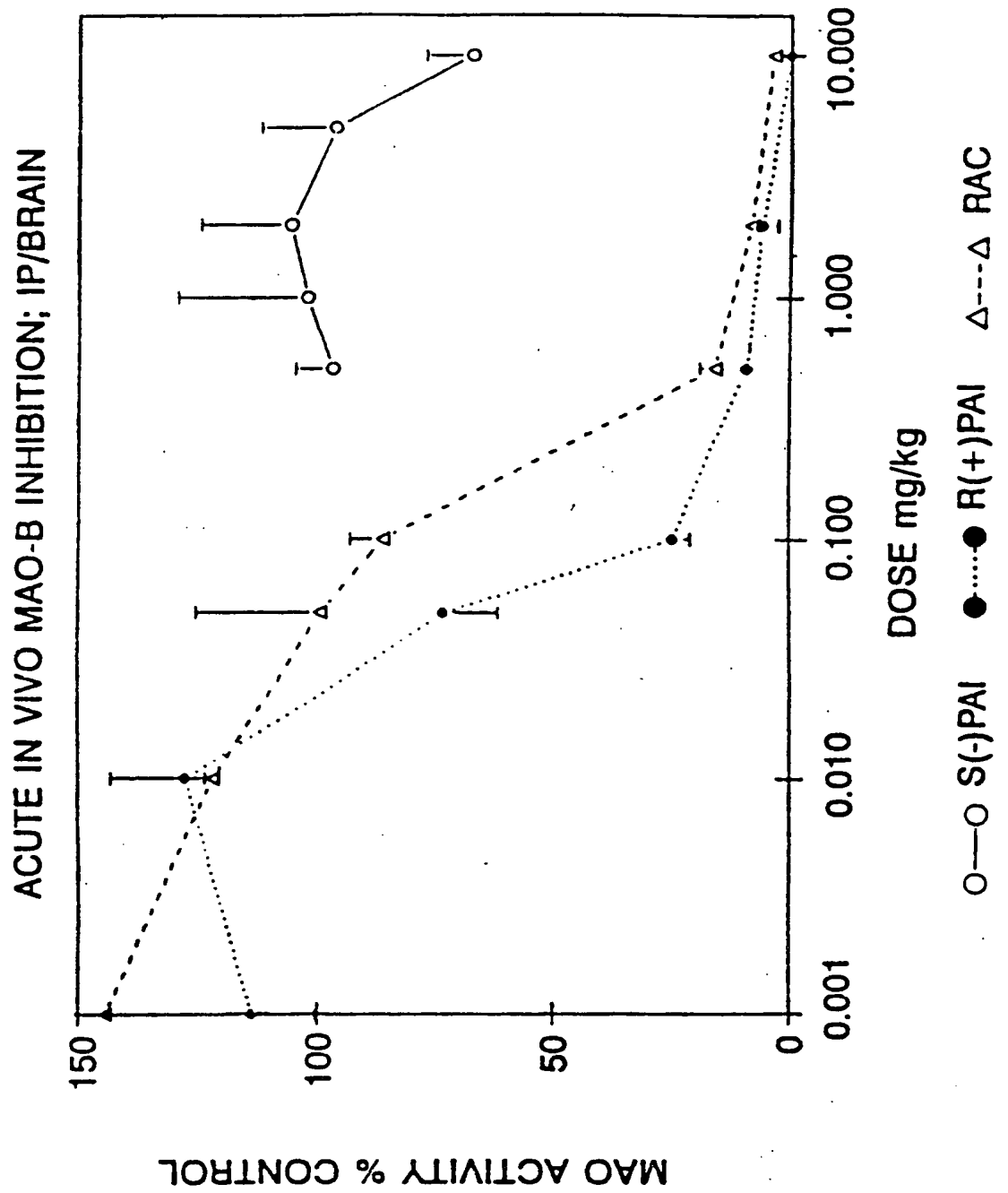
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FIGURE 4



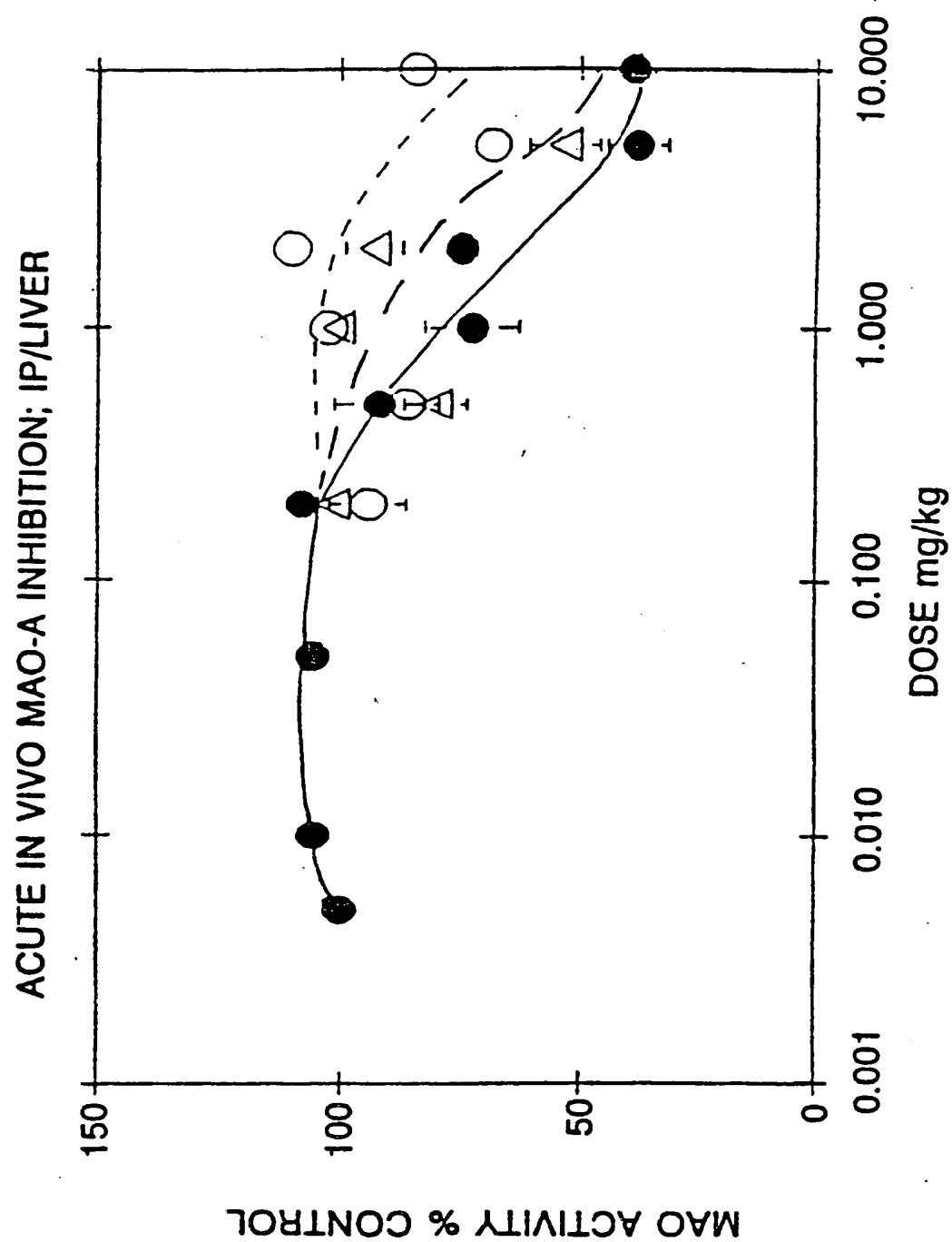
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FIGURE 5



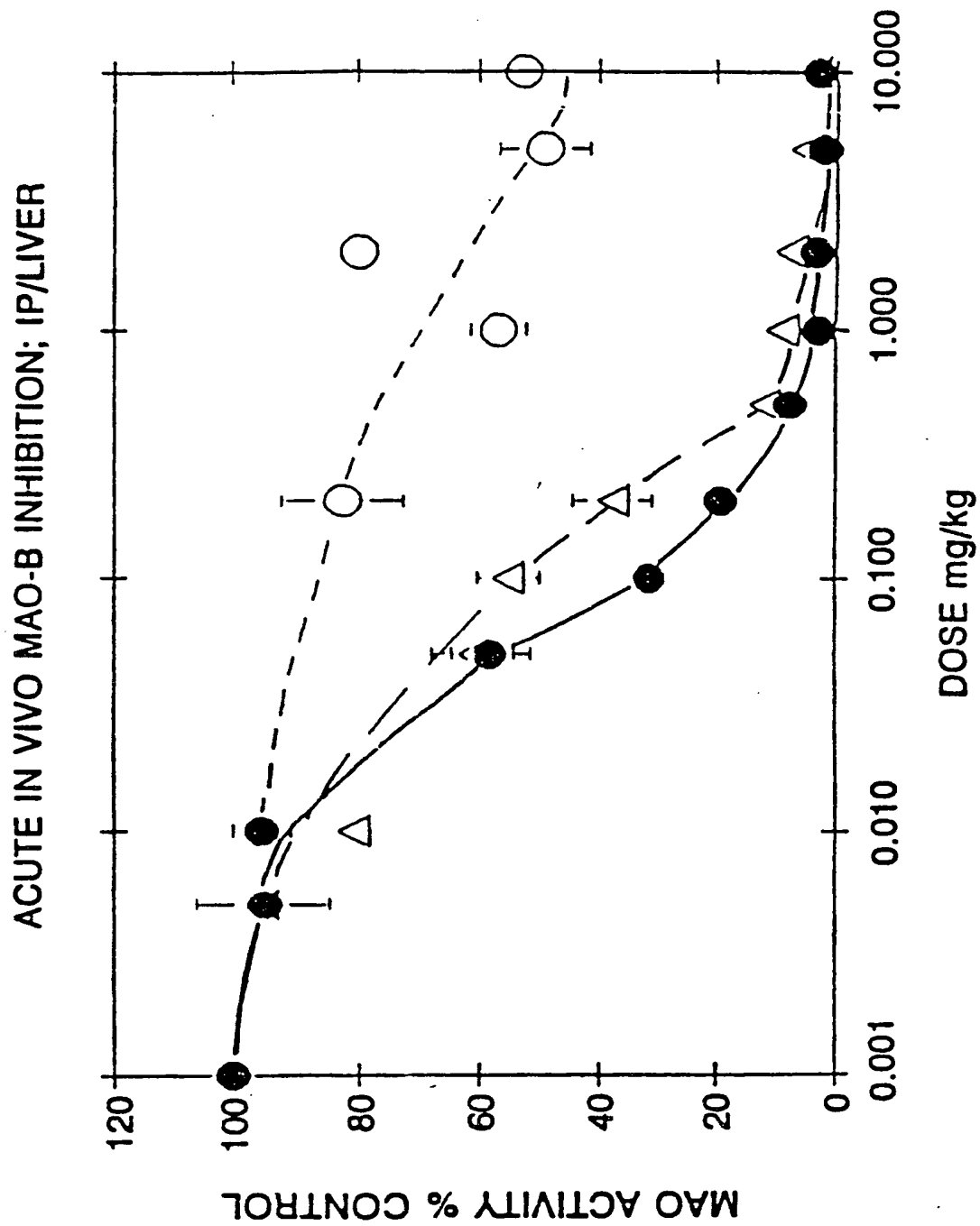
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FIGURE 6



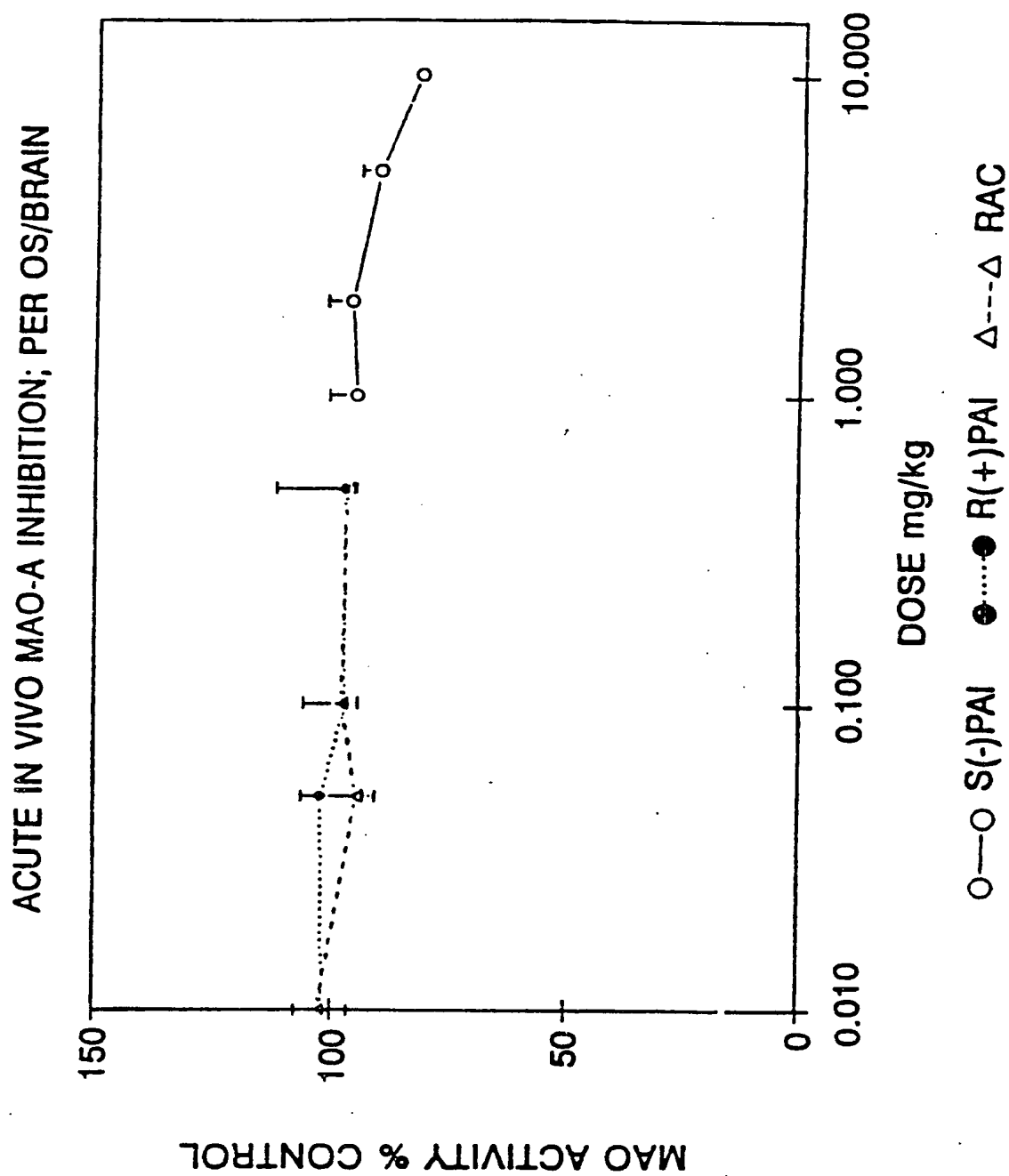
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FIGURE 7



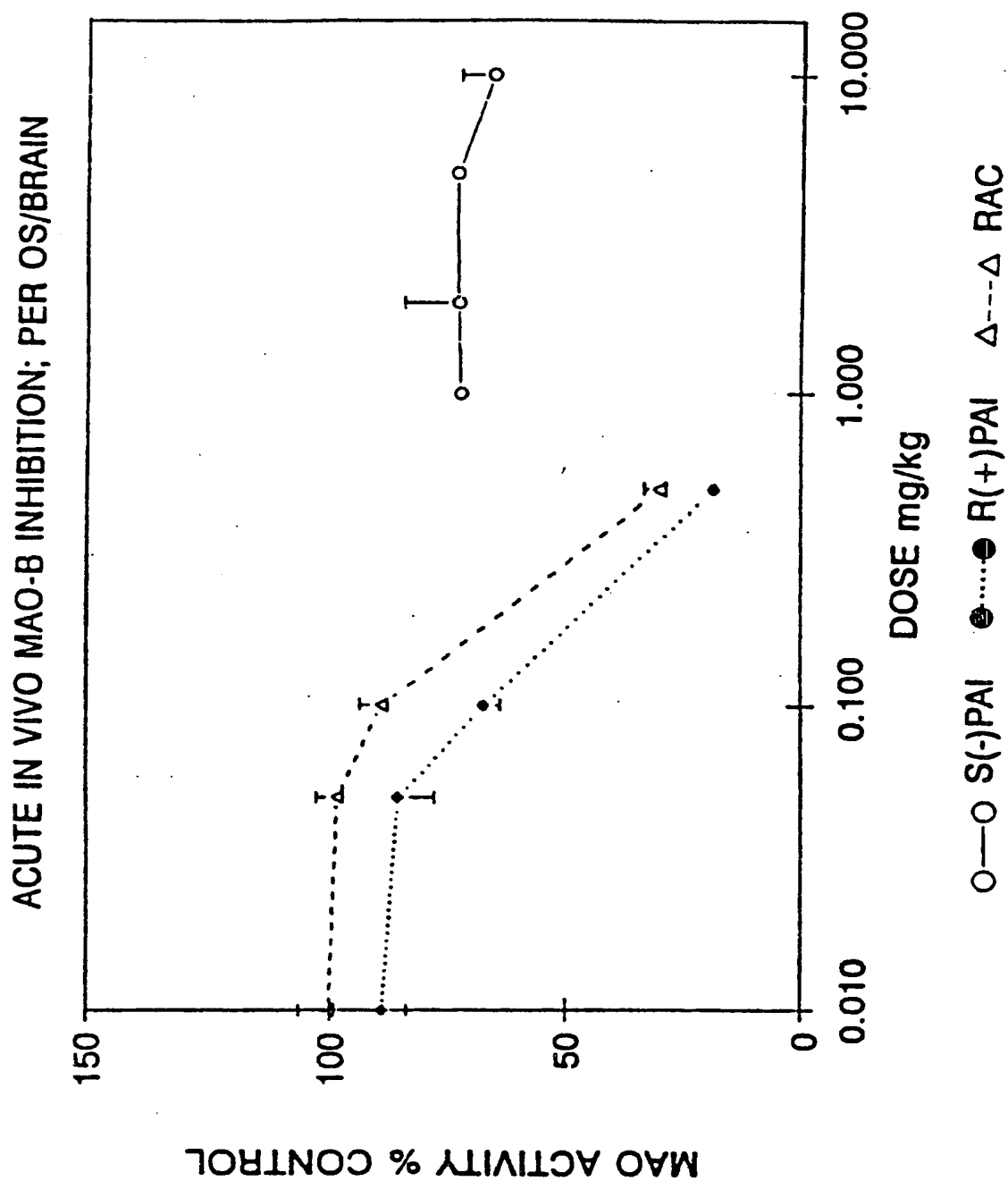
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FIGURE 8



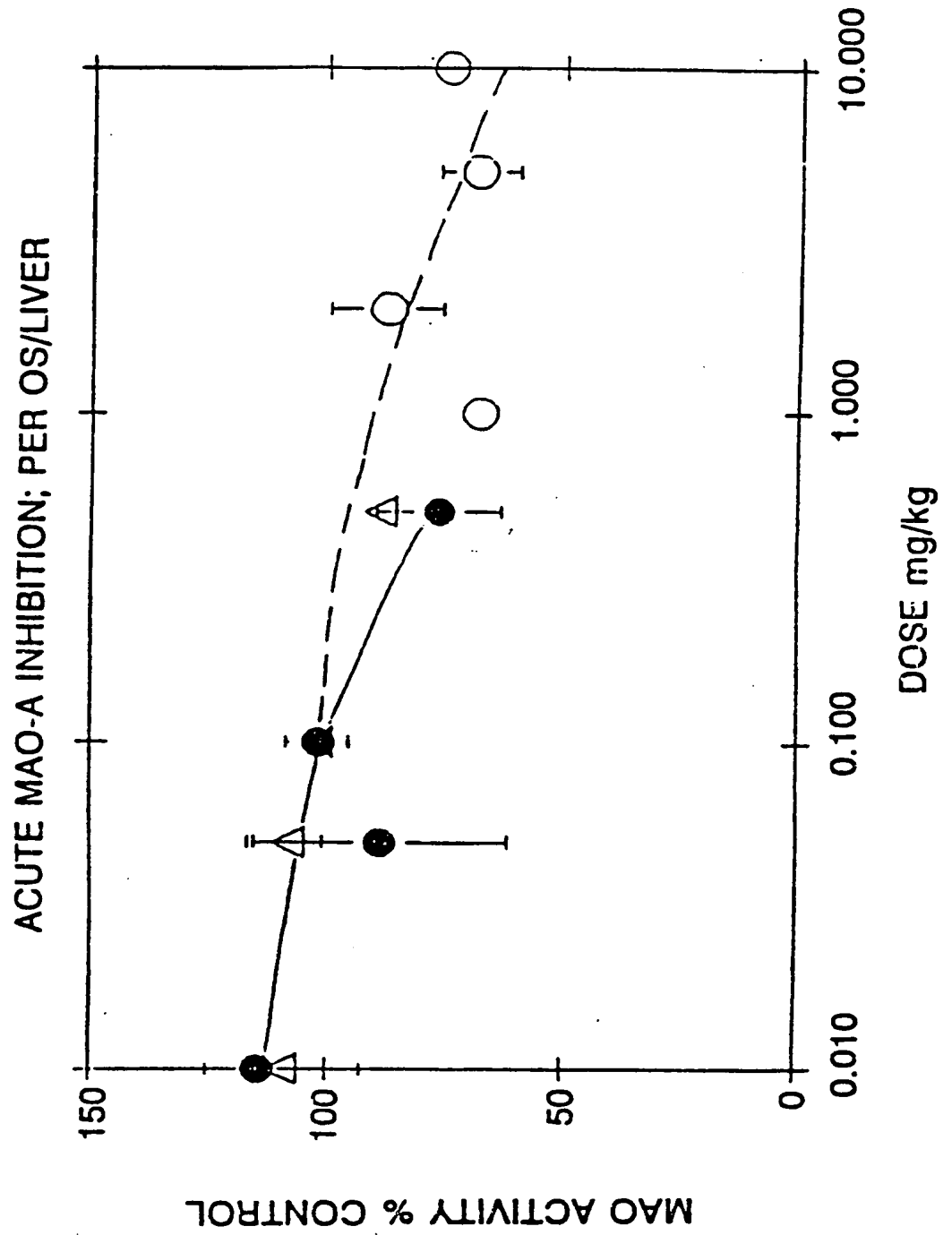
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FIGURE 9



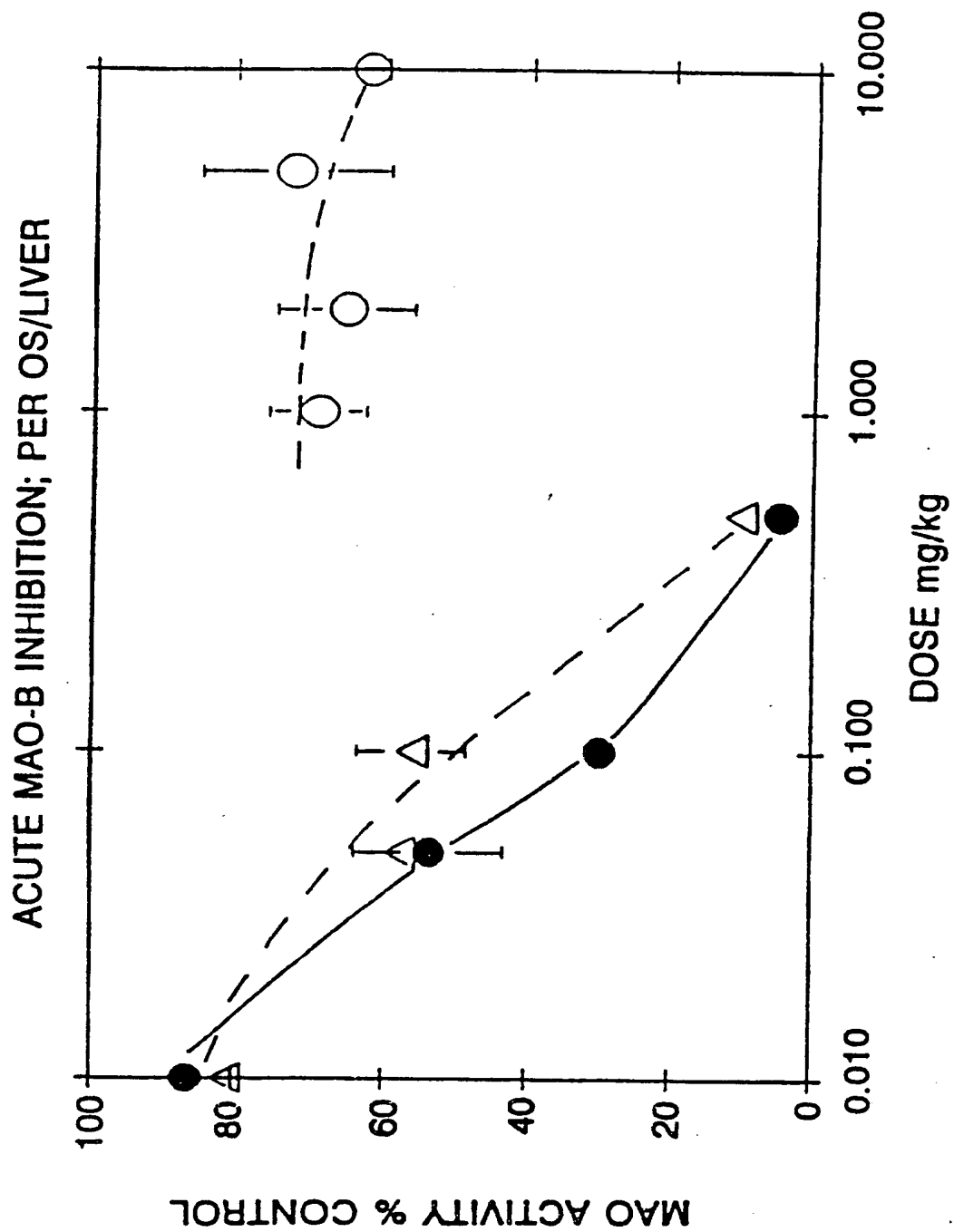
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FIGURE 10



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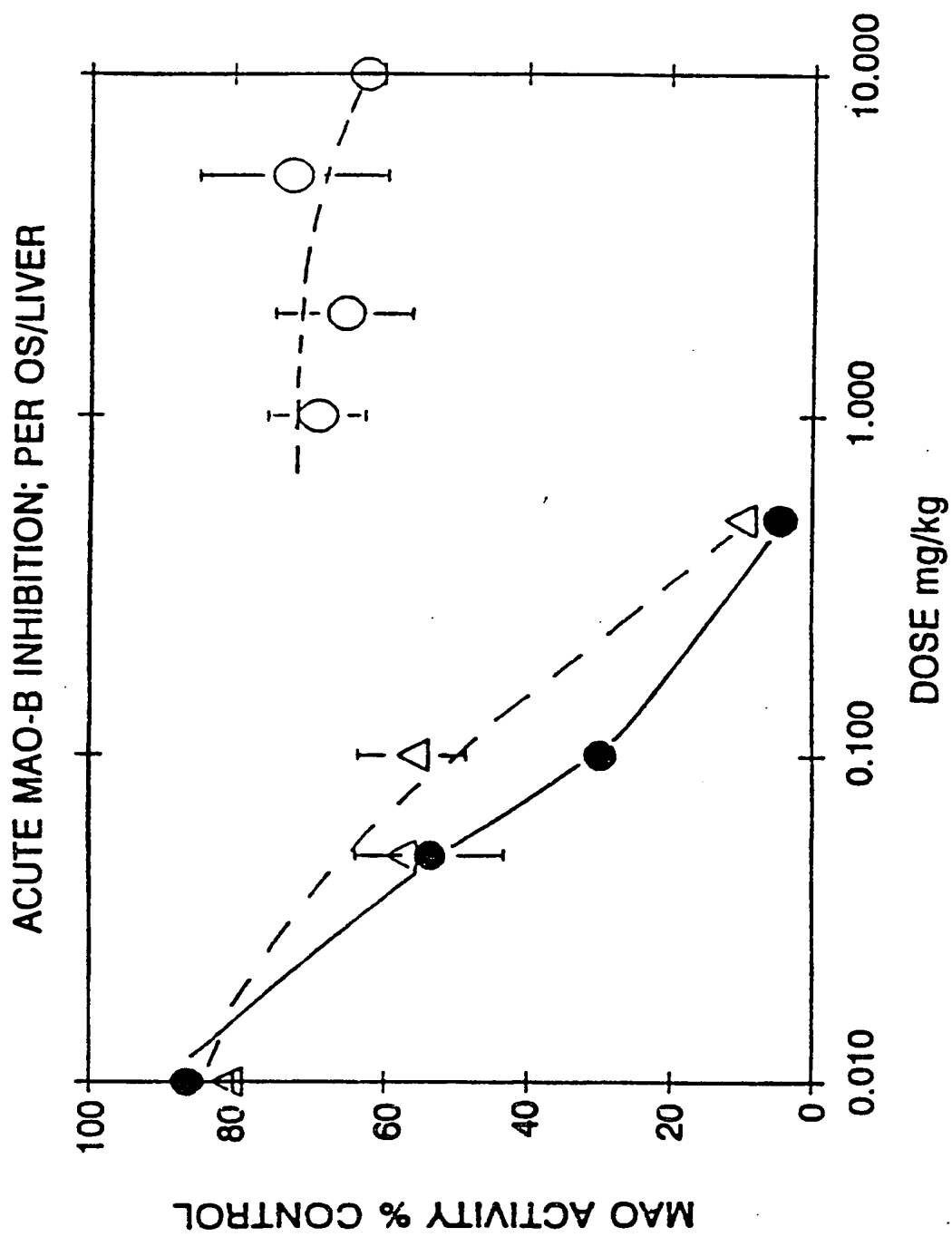
FIGURE 11





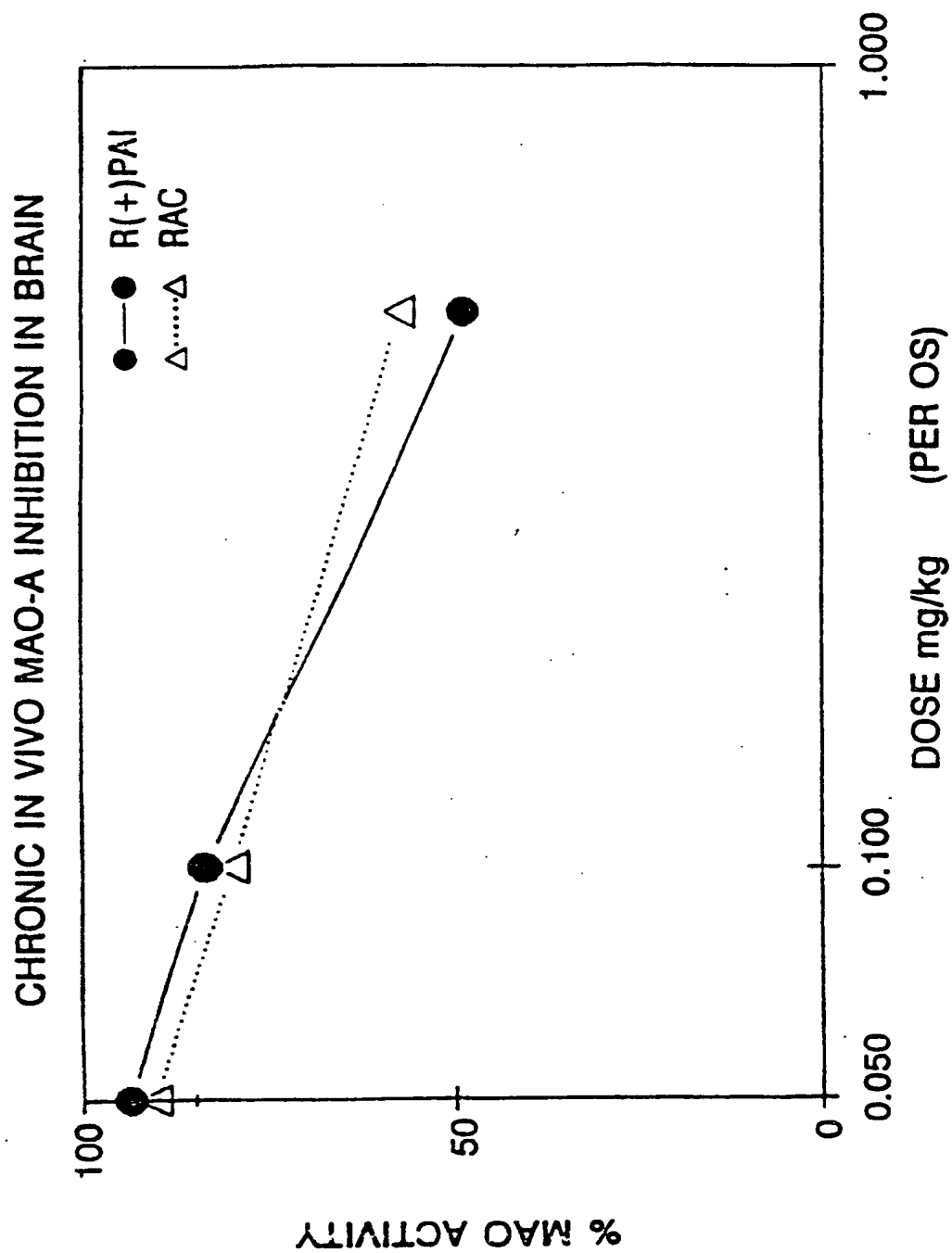
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FIGURE 11



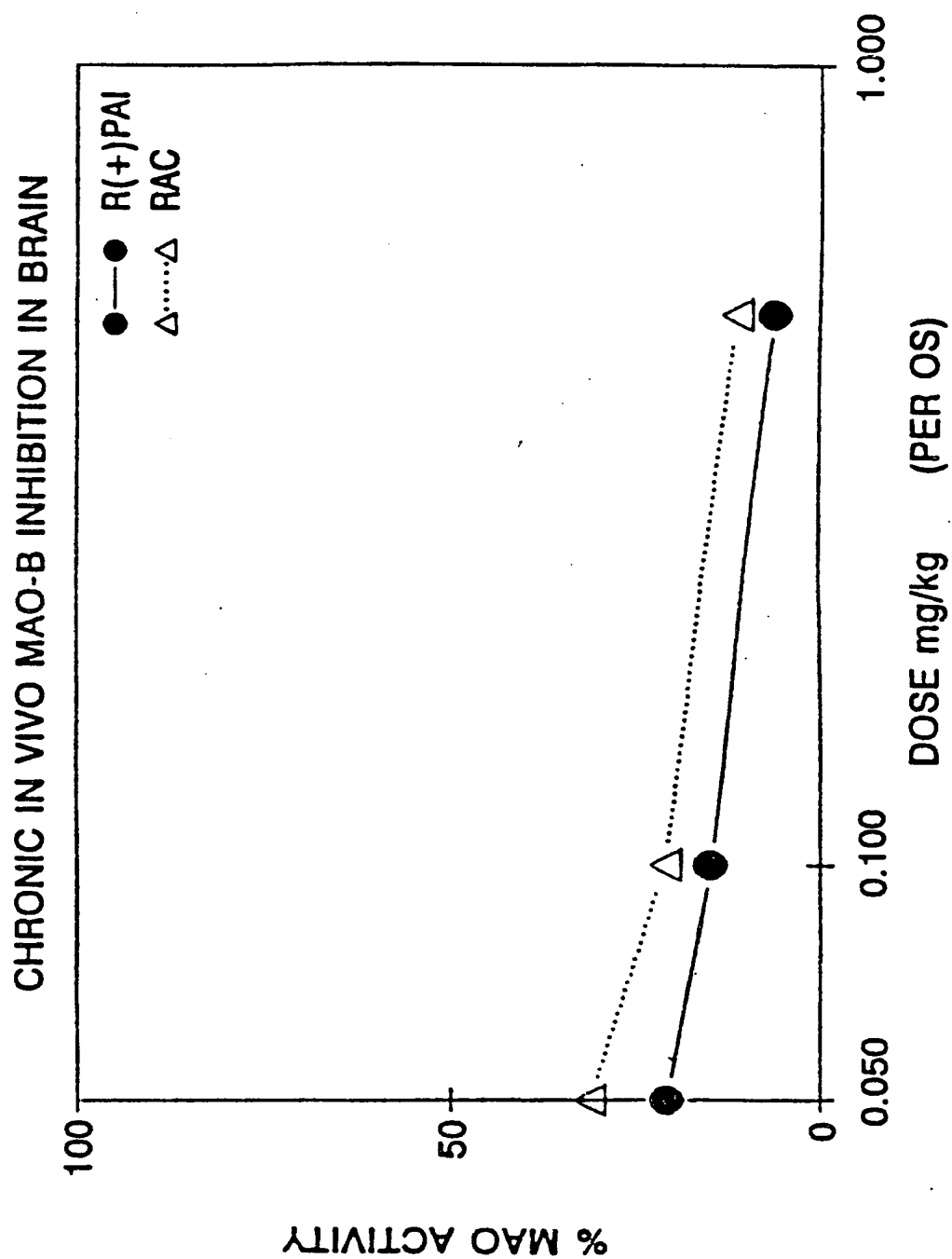
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FIGURE 12



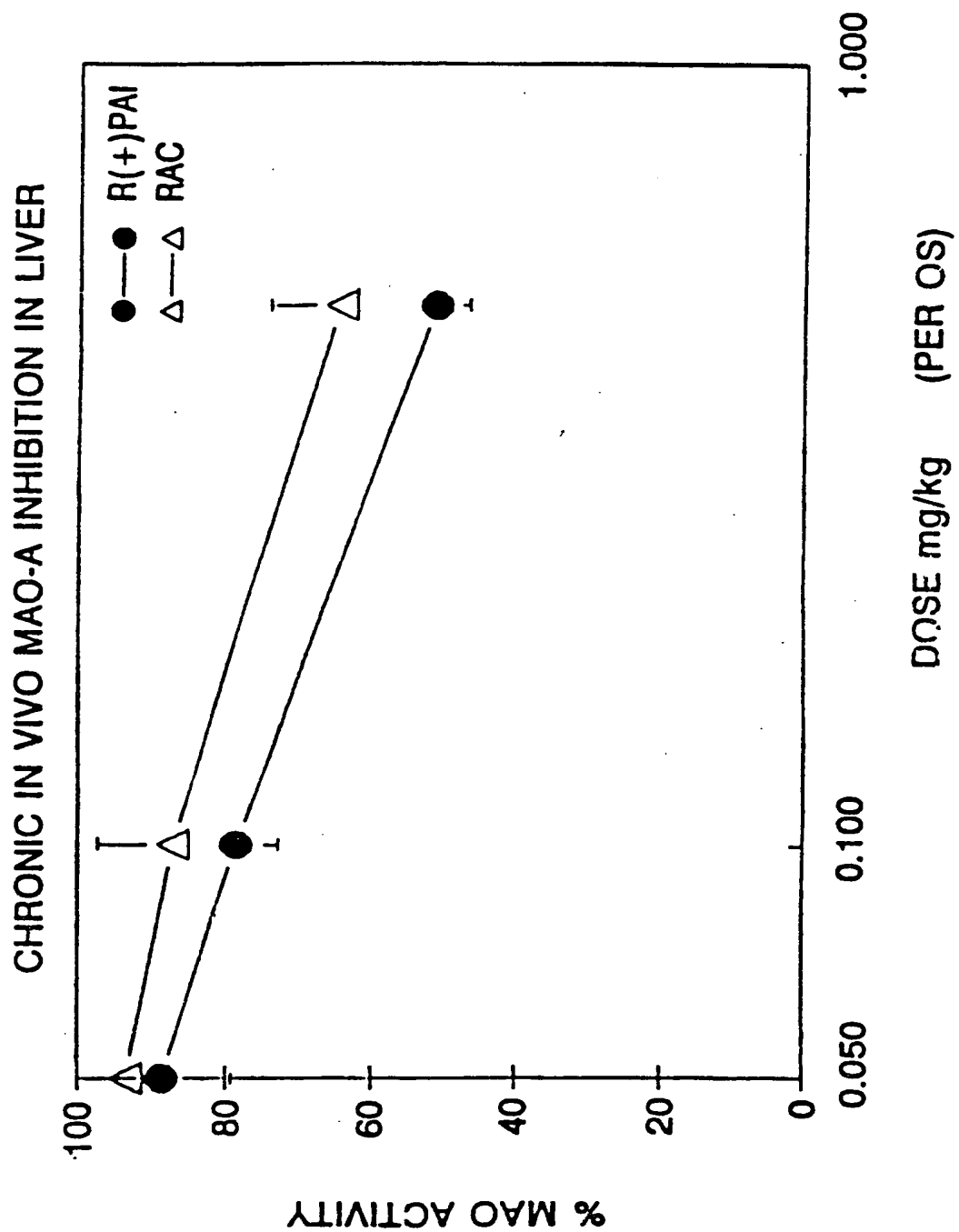
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FIGURE 13



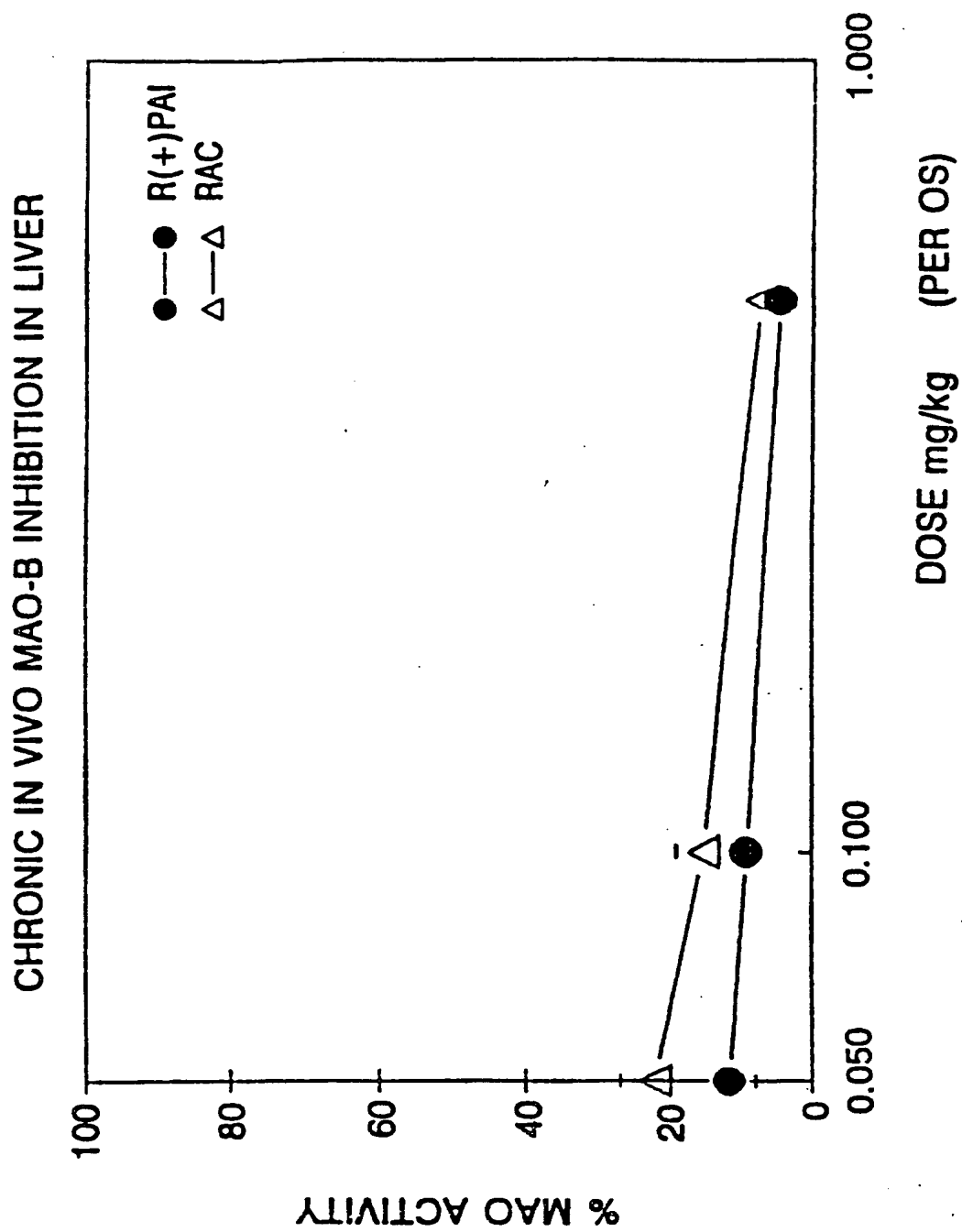
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FIGURE 14



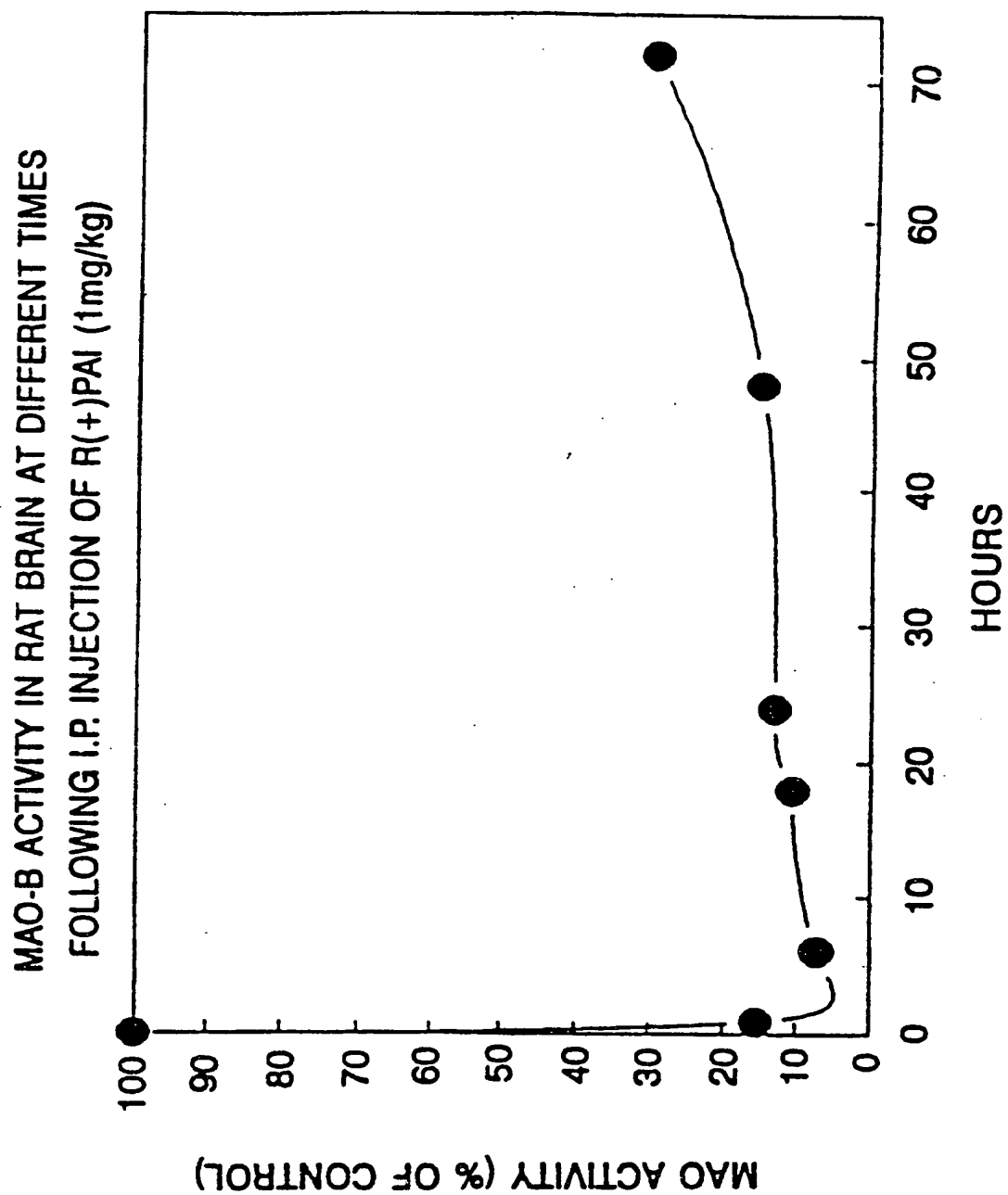
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FIGURE 15



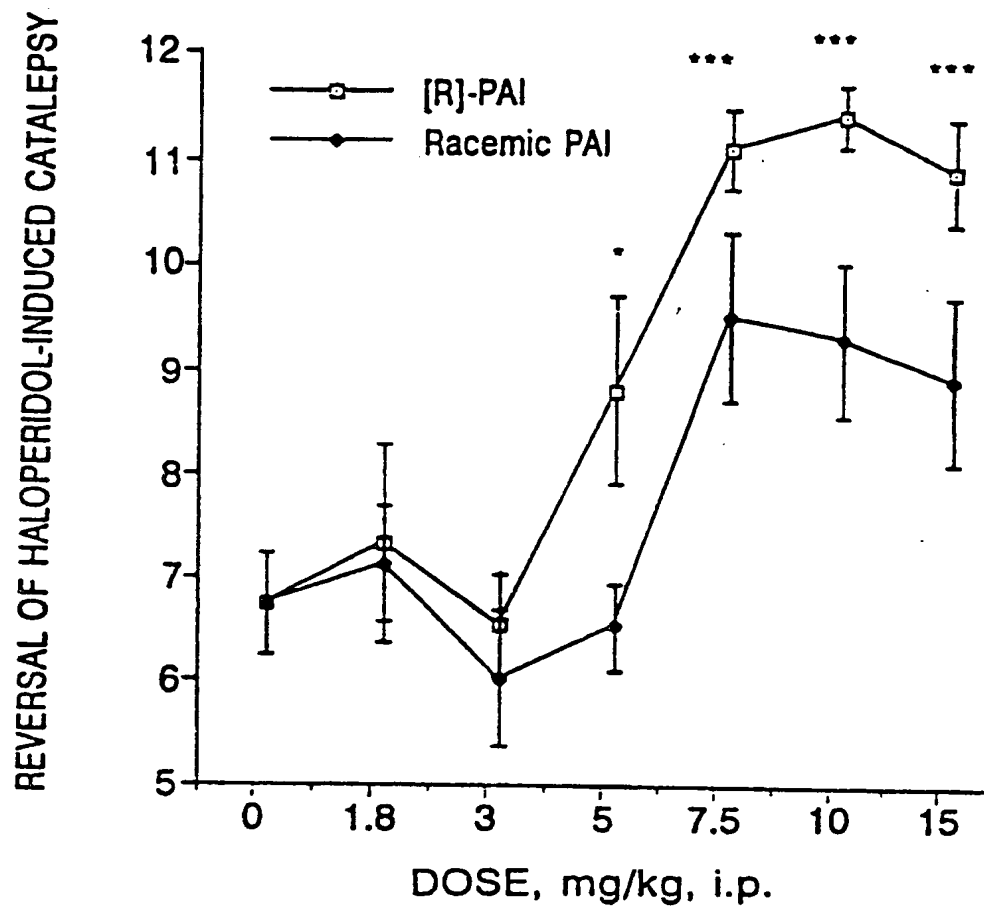
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FIGURE 16



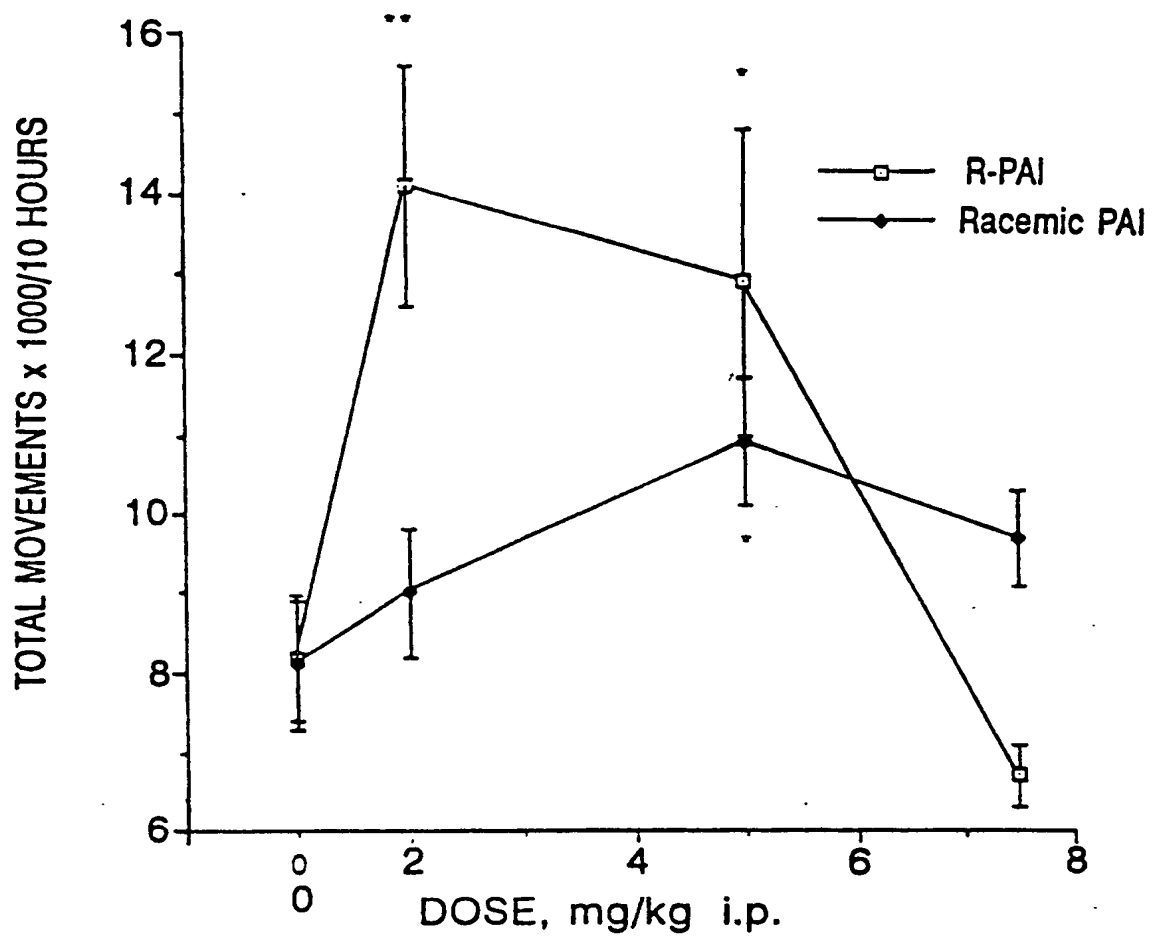
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FIGURE 17



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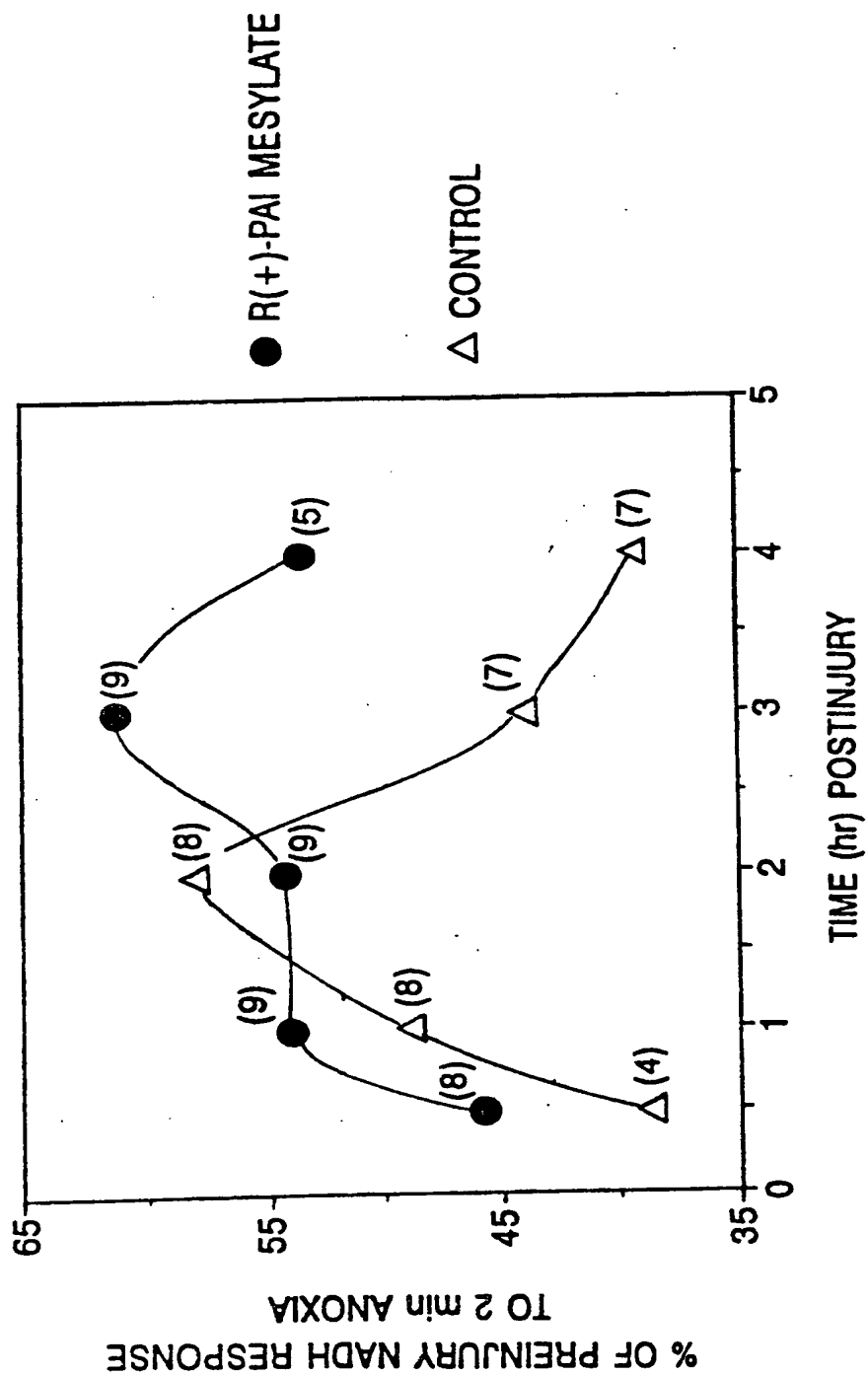
FIGURE 18





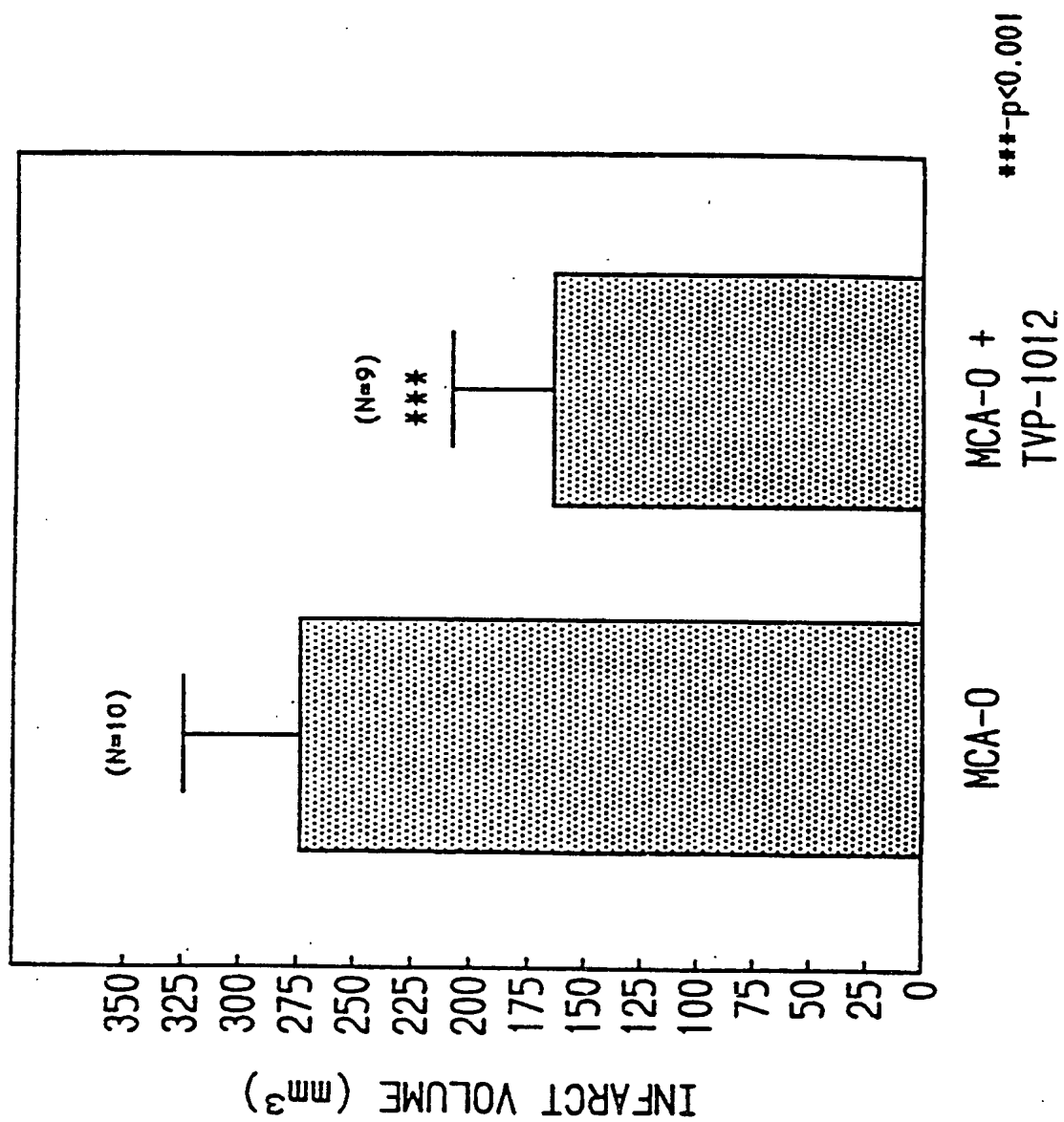
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FIGURE 19



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FIGURE 20



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FIGURE 21

